University of Alberta

Essential Fatty Acid Metabolism and Immune Function in Disease States Characterized by Immunosuppression and Abnormalities in Lipid Metabolism

by

Vera Christine Pratt ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial
fulfillment of the requirements for the degree of Doctor of Philosophy

in

Nutrition and Metabolism

Department of Agriculture, Food and Nutritional Science

Edmonton, Alberta

Spring 2001

National Library of Canada

Acquisitions and Bibliographic Services

395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque nationale du Canada

Acquisitions et services bibliographiques

395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence

Our file Notre référence

The author has granted a non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of this thesis in microform, paper or electronic formats.

The author retains ownership of the copyright in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

L'auteur a accordé une licence non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de cette thèse sous la forme de microfiche/film, de reproduction sur papier ou sur format électronique.

L'auteur conserve la propriété du droit d'auteur qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

0-612-60335-0

Canadä

University of Alberta

Library Release Form

Name of Author: Vera Christine Pratt

Title of Thesis: Essential Fatty Acid Metabolism and Immune Function in Disease States Characterized by Immunosuppression and Abnormalities in Lipid Metabolism

Degree: Doctor of Philosophy

Year this Degree Granted: 2001

Permission is hereby granted to the University of Alberta Library to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves all other publication and other rights in association with the copyright in the thesis, and except as herein before provided, neither the thesis nor any substantial portion thereof may be printed or otherwise reproduced in any material form whatever without the author's prior written permission.

Vera Pratt

Box 4328
Three Hills, Alberta
TOM 2NO

Date: Jan 29, 2001

University of Alberta

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and research for acceptance, a thesis entitled **Essential Fatty Acid Metabolism and Immune Function in Disease States Characterized by Immunosuppression and Abnormalities in Lipid Metabolism** submitted by Vera Christine Pratt in partial fulfillment of the requirements for the degree of Doctorate of Philosophy in Nutrition and Metabolism.

Dr. Catherine J. Field (Supervisor)

Dr. M. Thomas Clandinin (Committee Member)

Dr. Edward E. Tredget (Committee Member)

Dr. Vickie E. Baracos

Dr. Dominique Garrel

Date: Jan 12/01

This work is dedicated to

My parents
Who equipped me with
faith, strength, humility and a belief in myself
so that I could pursue wisdom

Chris Mazurak
Who added endurance to perseverance,
strength to determination,
courage to encouragement and joy
to the pursuit of wisdom

My nieces and nephews
For whom I wish
perseverance, courage and a belief in themselves
in their desire to pursue wisdom

Abstract

Critical illness results in a plethora of metabolic and immunologic abnormalities. Essential fatty acids have profound effects on metabolism and immune function. During stress states there are perturbations in fatty acid metabolism that may alter the fatty acid composition immune cell membranes which impact on their cellular function. These functional changes may place the patient at risk for infection. Changes in lipid composition of neutrophils and lymphocytes that occur following burn injury and during cancer have not been characterized previously. Immune changes that have been reported to occur after burn injury, and during cancer and chemotherapy have not been examined in relation to membrane composition. The objective of this thesis research was to demonstrate that alterations in fatty acid composition of immune cells contribute to functional changes observed in both the cell mediated and innate immune branches in disease states characterized by metabolic impairments and immunosuppression. The disease states that were investigated included infection, burn injury, high dose chemotherapy for breast cancer and cachexic cancer patients who were fed supplemental fish oil. Immune measures performed using peripheral blood immune cells included lymphocyte proliferation and cytokine production in response to mitogens, NK cell cytotoxicity, neutrophil oxidative burst and characterization of lymphocyte phenotypes that were associated with membrane phospholipid changes. For each disease state, the discussion will focus on the relationship of immune measures to the arachidonic acid content in the immune cell membranes. A relationship between the arachidonic acid content of neutrophil membranes and their oxidative burst response was confirmed by an

in vitro study. The implications of these findings in the design of nutritional intervention strategies aimed at up or down regulating specific immune functions will be discussed.

Acknowledgements

There are so many people I must acknowledge who contributed to the completion of this work. Dr. Catherine Field's enthusiasm for research started me on the research path. Her kindness and generosity as well as her critical reviews of each aspect of my thesis work, provided gentle encouragement for its successful completion. Dr. Tom Clandinin has an abounding knowledge and expertise in the area of fatty acid metabolism and has shown me several kindnesses and generosities throughout my Ph.D experience. His critical input and belief in my teaching abilities has served to be very valuable. I thank Dr. Tredget for providing insight into critical clinical issues and for being part of my supervisory committee. My committee's belief in the importance of this work was a source of enthusiasm during my Ph.D. I also thank Dr. Vickie Baracos (for the compliment of my life) and Dr. Dominique Garrel for serving on my thesis examining committee and Dr. Tapan Basu and Dr. Michael McBurney for serving on my candidacy examining committee.

Lindsay Robinson-Parsons has been an incredible blessing to me during my Ph.D work. The many "scientific" discussions, coffees and the friendship we have developed during our time together here will always be treasured. The Metabolic Units brought great joy to the extra-cirricular part of my Ph.D. David Ma was always ready and willing to help out and his computer skills are amazing. Thanks to Sue Goruk, who not only possesses tremendous technical skills but also served as a great friend. Other excellent technical assistance was provided by Nicola Wilson, Ping Li, Dorothy Rutkowski, Dr. Goh, Jack Demare and Gary Sedgewick. I thank all the graduate students in the Nutrition lab group who have enriched my Ph.D. experience. I also acknowledge the silent personal assistance of Lisa Makelki, Christie Hittel, Ginger Lunde, my parents and family who have all contributed to this work more than they know. I thank Chris Mazurak for waiting patiently and for being there when I needed him even when it seemed like I didn't.

I thank Heather Shankowsky, Tracey Dean, Kathryn Calder, Barb Tom, Gail Amiote, the nurses at the Fire Fighter's Burn Treatment Center and at Station 31 for providing the necessary assistance for completion of this work.

I thank the subjects in each of the human trials for showing me true strength and perseverance. Your contributions affirm my belief that this work is important and must continue.

I acknowledge the financial assistance of the American Oil Chemist's Society, NSERC and the families and trustees of both the Thomas H. Smouse Memorial Fellowship and the Izaak Walton Killam Memorial Fellowship.

TABLE OF CONTENTS

I. LIPIDS, IMMUNITY AND DISEASE

A. Dietary Fat and Lipid Biochemistry 1
B. Membranes: An Overview 3
C. Metabolism
  1. Macronutrient metabolism during stress 4
    a) Burn Injury 5
      (i) Protein Metabolism 5
      (ii) Carbohydrate Metabolism 6
      (iii) FatMetabolism 6
    b) Cancer and Cachexia 8
    c) Mediators in the Metabolic Response to Stress 11
D. The Immune System
  1. The Innate Immune System 13
    a) physical barriers 13
    b) macrophages 14
    c) natural killer cells 14
    d) neutrophils 15
  2. The Acquired Immune System 16
    a) T-lymphocytes 16
    b) B-cells 17
    c) Leukocyte Common Antigen (CD45) 18
    d) Flow Cytometry 18
  3. Mediators of the Immune System 19
  4. Wound healing 20
    a) immune system and wound healing 21
    b) Nutrition and wound healing 22
E. The Immune Response During Stress
  1. Burn injury 22

a) Innate Immunity 23
(i) Macrophages 23
(ii) Neutrophils 23
(iii) Natural Killer Cells 24
b) Acquired immunity 25
2. Infection 26
3. Cancer and its treatment 27
F. Immune Function and Fatty acids 30
1. Innate Immune cells 31
2. Lymphocytes 32
3. Mechanisms for Fatty Acid Effects on Immune Function 34
a) Fat as energy 34
b) Intracellular signals 35
c) Membrane Protein Activity 36
d) Eicosanoids 37
e) Cytokines 38
4. Neutrophils 38
5. Clinical trials 39
G. References 60

II. RESEARCH PLAN

A. Rationale 96
B. Objectives and Hypothesis 97
C. Chapter Layout 103
D. References 105

III. CHANGES IN NEUTROPHIL FATTY ACID COMPOSITION AND ITS RELATIONSHIP TO FUNCTION FOLLOWING BURN INJURY IN HUMANS

A. Introduction 106

B. Methods

1. Materials 107
2. Subjects 107
3. Dietary Intake 108
4. Sample Collection 108
5. Hemotological Analysis 109
6. Preparation of Neutrophils 109
7. Fatty acid Analysis 110
    a) Lipid Extraction 110
    b) Thin Layer Chromatography 110
    c) Methylation 111
    d) Gas Liquid Chromatography 111
8. Neutrophil Functional Parameters 111
9. Statistical Analysis 112

C. Results

1. Dietary intake 113
2. Neutrophil Concentration 113
3. Neutrophil Functional Parameters 114

4. Fatty Acid Composition of Neutrophils

    a) 18:2n-6 and 20:4n-6 114
    b) Total n-6, n-3 and n-6/n-3 Ratio 114
    c) Total SFA, MUFA and PUFA 115
D. Discussion 116
E. References 135

III. ALTERATIONS IN LYMPHOCYTE FUNCTION AND RELATION TO PHOSPHOLIPID COMPOSITION FOLLOWING BURN INJURY

A. Introduction 141

B. Methods

1. Materials 142
2. Subjects 142
3. Preparation of Lymphocytes 143

4. Mitogenic Responses of Immune Cells 143
5. Mononuclear Cell Phenotyping 144
6. NK Cell Cytotoxicity 145
7. Cytokine Production 146
8. Cytokine Determination 146
9. Fatty Acid Analysis 147
10. Statistical Analysis 148

C. Results

1. Dietary Intake 148
2. Phenotypes in Peripheral Blood 148
3. Estimated Proliferation 148
4. NK Cell Cytotoxicity 149
5. Cytokine Production 149
6. Phospholipid Analysis 149
D. Discussion 150
E. References 169

IV. FATTY ACID CONTENT OF PLASMA LIPIDS AND ERYTHROCYTE PHOSPHOLIPIDS ARE ALTERED FOLLOWING BURN INJURY

A. Introduction 175
B. Methods 176
1. Serum Collection 176

2. Fatty Acid Analysis

a) Lipid Extraction 176
b) Thin Layer Chromatography 176
c) Saponification 177
d) Methylation 177
e) Gas-Liquid Chromatography 177
3. Statistical Analysis 178

C. Results

1. Dietary Intake 178

2. Plasma Total Phospholipids 178
3. Major Phospholipid Fractions 179
4. Cholesteryl Esters 179
5. Triglycerides 180
6. Erythrocyte Phospholipids 180

D. Discussion 180

E. References 195

V. *STAPHYLOCOCCUS AUREUS* INFECTION REDUCES NATURAL KILLER CELL CYTOTOXICITY AND STIMULATES IMMUNE FUNCTION FOLLOWING SURGERY

A. Introduction 200

B. Methods

1. Materials 201
2. Animals and Surgical Procedures 202
3. Preparation of Lymphocytes 203
4. Mitogenic Responses of Immune Cells 203
5. Mononuclear Cell Phenotyping 204
6. NK Cell Cytotoxicity 205
7. Neutrophil Oxidative Burst 205
8. Statistical Analysis 205

C. Results

1. Weight Loss 206
2. Mitogenic Responses of Splenocytes 206
3. Phenotyping 207
4. NK Cytotoxicity 207
5. Neutrophil Response to Stimulation with PMA 208

D. Discussion 208

E. References 219

VI. STEM CELL HARVEST AND HIGH DOSE CHEMOTHERAPY IN WOMEN WITH BREAST CANCER CHANGES NEUTROPHIL FATTY ACID COMPOSITION AND IMMUNE FUNCTION

A. Introduction 223

B. Methods

1. Subjects 224
2. Sample Collection 225
3. Identification of Immune Cell Subsets 225
4. Neutrophil Function 225
5. Neutrophil Phospholipid Analysis 226
6. Plasma Lipid Analysis 226
7. Statistical Analysis 226

C. Results

1. Subjects 226
2. Cell Counts 227
3. Immune Cell Phenotypes 227
4. Neutrophil Functional Parameters 227
5. Neutrophil PL Analysis 228
6. Plasma PL Analysis 228

D. Discussion 228

E. References 240

VII. IMPROVEMENTS IN IMMUNE FUNCTIONS AND ALTERATIONS IN PLASMA AND NEUTROPHIL PL COMPOSITION AFTER SUPPLEMENTATION WITH FISH OIL IN WEIGHT LOSING CANCER PATIENTS

A. Introduction 243

B. Methods

1. Materials 244
2. Subjects 244

3. Preparation of Neutrophils 244
4. Phospholipids Fatty Acid Analysis 245
5. Neutrophil Oxidative Burst 245
6. Mononuclear Cell Phenotyping 246
7. Statistical Analysis 246

C. Results

1. Subjects 246
2. Fatty Acid Composition of Plasma PL 247
3. Fatty Acid Composition of Neutrophil PL 247
4. Neutrophil Function 247
5. Lymphocyte Phenotypes 248
D. Discussion 248
E. References 261

VIII. CHANGES IN MEMBRANE COMPOSITION ARE ASSOCIATED WITH CHANGES IN OXIDATIVE BURST IN AN *IN VITRO* MODEL

A. Introduction 265

B. Methods

1. Materials 266
2. Ethics Approval 266
3. Preparation of Fatty Acids 266
4. Fatty Acid Incorporation 267
5. Oxidative Burst 267
6. Lipid Extraction and Phospholipids Analysis 268
7. Statistical Analysis 268
C. Results 268
1. PL Fatty Acid Composition 268
2. Relationship of 20:4n-6 to Changes in the Oxidative Burst 269
D. Discussion 270
E. References 276

IX. GENERAL SUMMARY AND DISCUSSION

A. General Summary 278
B. Discussion and Future Directions 288
    1. The Effects of Burn Injury on Fatty Acid Metabolism And Immune Function 288
    2. Phospholipids 291
    3. Surgery and Infection 292
    4. High Dose Chemotherapy 292
    5. Cancer Cachexia 293
C. Summary and Conclusions 294
D. References 296

Appendix III 300
Appendix IV 305
Appendix V 310
Appendix VII 318
Appendix VIII 320
Appendix IX 321

List of Tables

CHAPTER I

Table I.1 Infections and the Types of Immune Responses Most effective in Eliminating them 56

Table I.2 Clinical Trials Investigating the Supplementation of Fatty Acids in Burns and Cancer 57-59

CHAPTER III

Table III.1 Characteristics of Patients Included in the Study and Mean Dietary Intake 122

Table III.2A Nutritional Composition of Nitro-Pro 123

Table III.2B Vitamin and Mineral Composition of Nitro-Pro 124

Table III.3 Fatty Acid Composition of Nitro-Pro 125

Table III.4 Composition of Pro-Mod Protein Supplement 126

Table III.5 Effect of Burn Injury on Size of Neutrophils before and After Stimulation with PMA 127

Table III.6 Effect of Burn Injury on granularity of Neutrophils Before and After stimulation with PMA 128

Table III.7 Effect of Burn Injury on 20:4n-6 content of Neutrophil PL Fractions 129

Table III.8 The 18:2n-6/20:4n-6 Ratio in Neutrophil PL 130

Table III.9 Fatty Acid composition of the PC Fraction of Neutrophils 131

Table III.10 Fatty Acid composition of the PI Fraction of Neutrophils 132

Table III.11 Fatty Acid composition of the PS Fraction of Neutrophils 133

Table III.12 Fatty Acid composition of the PE Fraction of Neutrophils 134

CHAPTER IV

Table IV.1 Specificities of Monoclonal Antibodies 158
Table IV.2 Three Colour Combinations of Monoclonal Antibodies 159
Table IV.3 Lymphocyte Subsets Following Burn Injury 160
Table IV.4 Relative Percents of CD4 and CD8 Cells Expressing Common Leukocyte Antigen 161
Table IV.5 Relative Percents of CD4 and CD8 Cells Expressing Activation Markers 162
Table IV.6 Proliferation of Lymphocytes in Response to Mitogens 163
Table IV.7 Fatty Acid composition of the PC Fraction of Lymphocytes 164
Table IV.8 Fatty Acid composition of the PS Fraction of Lymphocytes 165
Table IV.9 Fatty Acid composition of the PI Fraction of Lymphocytes 166
Table IV.10 Fatty Acid composition of the PE Fraction of Lymphocytes 167
Table IV.11 The 18:2n-6/20:4n-6 Ratio of Lymphocyte PL 168

CHAPTER V

Table V.1 Fatty Acid Composition of Plasma Total PL Following Burn Injury Expressed in Percent of Total Fatty Acids 186
Table V.2 Fatty Acid Composition of Plasma Total PL Following Burn Injury Expressed in μg/ml plasma 187
Table V.3 Fatty Acid Composition of Major Plasma PL Fractions Following Burn Injury 188
Table V.4 Fatty Acid Composition of Plasma Total CE Following Burn Injury Expressed in Percent of Total Fatty Acids 189
Table V.5 Fatty Acid Composition of Plasma CE Following Burn Injury Expressed in μg/ml plasma 190
Table V.6 Fatty Acid Composition of Plasma TG Following Burn Injury Expressed in Percent of Total Fatty Acids 191
Table V.7 N-3 Fatty Acids in the PC and PE Fractions of Erythrocytes 192

Table V.8 Fatty Acid composition of PC and PE Fractions of Erythrocytes 193
Table V.9 Fatty Acid composition of the PS Fraction of Erythrocytes 194

CHAPTER VI

Table VI.1 Specificities of the Monoclonal Antibodies Used to Identify Guinea Pig Immune Cell Subsets 215
Table VI.2 Effect of Surgery, Infection and Acticoat on Splenocyte Mitogenic Responses 216
Table VI.3 Effect of Surgery, Infection and Acticoat on Prescalpular Immune Subsets 217
Table VI.4 Effect of Surgery, Infection and Acticoat on Neutrophil Oxidative Burst 218

CHAPTER VII

Table VII.1 Lymphocyte Subsets Before and After High Dose Chemotherapy 236
Table VII.2 Fatty Acids in the PC and PS Fraction of Neutrophil PL 237
Table VII.3 20:4n-6 in Major PL Fractions Before and After High Dose Chemotherapy 238
Table VII.4 Fatty acids in Plasma Before and After High Dose Chemotherapy 239

CHAPTER VIII

Table VIII.1 Three Colour Combinations of Monoclonal Antibodies used to Identify Lymphocyte Subsets 253
Table VIII.2 Characteristics of Subjects Randomly Allocated to Receive Fish Oil 254

Table VIII.3 Fatty Acid Composition of plasma PL Expressed as a Percent of Total Fatty Acids 256

Table VIII.4 Fatty Acid Composition of Major PL Fractions in Neutrophils Before and After 14 days Of Fish Oil Supplementation 257

Table VIII.5 Oxidative Burst of Neutrophils Before and After 14 Days of Fish Oil Supplementation with Fish Oil or Placebo 258

Table VIII.6 Granularity of Neutrophils Before and After 14 Days of Fish Oil Supplementation with Fish Oil or Placebo 259

Table VIII.7 Lymphocyte Subsets Characterized by Monoclonal Antibodies Before and After Supplementation with Fish Oil 260

CHAPTER IX

Table IX.1 Fatty Acids Used in the Cell Culture Experiments 272

Table IX.2 Fatty Acid Composition of Neutrophils Before and Changes in Fatty acids After Stimulation with PMA 273

Table IX.3 Oxidative Burst of Neutrophils Cultured with Fatty Acids 274

Table IX.4 Total Neutrophil PL in Human Trials 275

APPENDICES

Table III.AP1 Fatty Acid Composition of the PC Fraction of Neutrophils Following Burn Injury 300

Table III.AP2 Fatty Acid Composition of the PI Fraction of Neutrophils Following Burn Injury 301

Table III.AP3 Fatty Acid Composition of the PS Fraction of Neutrophils Following Burn Injury 302

Table III.AP4 Fatty Acid Composition of the PE Fraction of Neutrophils Following Burn Injury 303

Table III.AP5 **Fatty Acid Composition of Neutrophils Isolated From Healthy Subjects** **304**

Table IV.AP1 **Fatty Acid Composition of the PC Fraction of Lymphocytes Following Burn Injury** **305**

Table IV.AP2 **Fatty Acid Composition of the PS Fraction of Lymphocytes Following Burn Injury** **306**

Table IV.AP3 **Fatty Acid Composition of the PI Fraction of Lymphocytes Following Burn Injury** **307**

Table IV.AP4 **Fatty Acid Composition of the PE Fraction of Lymphocytes Following Burn Injury** **308**

Table IV.AP5 **Fatty Acid Composition of Lymphocytes Isolated from Healthy Subjects** **309**

Table V.AP1 **Fatty Acid Composition of Plasma PL Following Burn Injury Expressed as μg/ml** **310**

Table V.AP3 **Fatty Acid Composition of Plasma CE Following Burn Injury Expressed as μg/ml** **311**

Table V.AP4 **Fatty Acid Composition of Plasma PL, CE and TG Isolated from Healthy Subjects** **312**

Table V.AP5 **Fatty Acid Composition of the PC fraction of Erythrocytes Following Burn Injury** **314**

Table V.AP6 **Fatty Acid Composition of the PE fraction of Erythrocytes Following Burn Injury** **315**

Table V.AP7 **Fatty Acid Composition of the PS fraction of Erythrocytes Following Burn Injury** **316**

Table V.AP8 **Fatty Acid Composition of the PI fraction of Erythrocytes Following Burn Injury** **317**

Table VII.AP1 **Fatty Acid Composition of Plasma PL, CE and TG Isolated before and After HDCT expressed in μg/ml Plasma** **318**

Table VII.AP1 **Fatty Acid Composition of Plasma PL, CE and TG Isolated before and After HDCT expressed in % Fatty Acids** **319**

Table VIII.AP1 **Fatty Acid Content of Plasma PL Before and After** **320**

Supplementation with Fish Oil and Placebo

Table IX.AP1 Number of Neutrophils Isolated from Ficoll-Hypaque Gradient Centrifugation after Fatty Acid Incubation with Whole Blood 321

List of Figures

CHAPTER I

Figure I.1 Conversion of linoleic and linolenic acid into other fatty acids by elongase and desaturase pathways 44
Figure I.2 Synthesis of prostaglandins from arachidonic acid 45
Figure I.3 Biological Membrane 46
Figure I.4 Major metabolic changes that occur with the stress response versus starvation 47
Figure I.5 The Effects of Proinflammatory cytokines on various tissues in the body 48
Figure I.6 The Innate Immune System 49
Figure I.7 Activation of the NADPH oxidase enzyme complex 50
Figure I.8 Analysis of the Respiratory Burst using Flow cytometry 51
Figure I.9 The Acquired Immune System 52
Figure I.10 Interactions between the Innate and Acquired Immune Cells 53
Figure I.11 The Role of Immune Cells in Wound Healing 54
Figure I.12 Lipids as Intracellular Messengers 55

CHAPTER III

Figure III.1 Effect of time Post Burn Injury on Oxidative Burst Ratio of Neutrophils 120
Figure III.2 Effect of time Post Burn Injury on Total 20:4n-6 Content of Neutrophil PL 121

CHAPTER IV

Figure IV.1 Effect of time Post Burn Injury on Natural Killer Cell Cytotoxicity 155
Figure IV.2 20:4n-6 Content in Lymphocyte PL Following Burn Injury 156

Figure IV.3 The n-6/n-3 Ratio in Lymphocyte PL Following Burn Injury 157

CHAPTER V

Figure V.1 Total Plasma PL of Subjects Following Burn Injury 184
Figure V.2 Total Plasma CE of Subjects Following Burn Injury 185

CHAPTER VI

Figure VI.1 Effect of Surgery and Infection on Guinea Pig Splenocytes Phenotypes 213
Figure VI.2 Effect of Surgery and Infection on Natural Killer Cell Cytotoxicity 214

CHAPTER VII

Figure VII.1 Schematic of the High Dose Chemotherapy Procedure 232
Figure VII.2 The CD4/CD8 Ratio at Harvest and Discharge 233
Figure VII.3 Effect of High Dose Chemotherapy on Oxidative Burst of Neutrophils 234
Figure VII.4 Effect Of High Dose Chemotherapy on the Size Index of Neutrophils 235

CHAPTER VIII

Figure VIII.1 Percent of EPA and DHA in Plasma Before and After 14 Days Supplementation with Fish Oil 252

APPENDICES

Figure IX.AP1 Measurement of [^{3}H] Thymidine in Supernatants of Cultures After Incubation with Fatty Acids 319

Figure IX.AP2 Oxidative Burst of Neutrophils Cultured in Fatty Acids 320

List of Abbreviations

°C: Degrees Celsius

18:2n-6: Linoleic acid

20:4n-6: Arachidonic acid

ANOVA: Analysis of variance

ANSA: 8-anilino-1-naphthalene-sulfonic acid

APC: Antigen presenting cell

APR: Acute phase response

ARDS: Acute respiratory distress syndrome

BF_3: Boron trifluoride

BSA: Bovine serum albumin

cAMP: Cyclic AMP

CBC: Complete blood count

CE: Cholesteryl ester

Con A: Concanavalin A

CCM: Complete culture media

Cfu: colony forming units

CTL: Cytotoxic T lymphocyte

D: day

DHA: Docosahexaenoic acid

DHR: Dihydrorhodamine

DMSO: Dimethyl sulfoxide

Dpm: decays per minute

DTH: Delayed type hypersensitivity

EPA: Eicospentaenoic acid

FCS: Fetal calf serum

FITC: Fluoroscein isothiocyanate

FSC: Forward scatter

GALT: Gut associated lymphoid tissue

GI: Gastrointestinal

Gm: grams

HBSS: Hank's balanced salt solution

HDCT: High dose chemotherapy

IFN: Interferon

Ig: Immunologlobulin

IL: Interleukin

Kcal: Kilocalorie

Kg: kilogram

KJ: Kilojoule

KRH: Kreb's Ringer Hepes

LPL: Lipoprotein lipase

LPS: Lipopolysaccharide

mAb: Monoclonal antibody

MALT: Mucosal associated lymphoid tissue

MHC: Major histocompatibility complex

MOF: Multiple organ failure

NK: Natural killer

PBMC: Peripheral blood mononuclear cell

PC: Phosphatidylcholine

PE: Phosphatidylethanolamine

rPE: Phycoerthyrin

PGE$_2$: Prostaglandin E2

PHA: Phytohemmatogluttinin

PI: Phosphatidylinositol

PKC: Protein kinase C

PL: Phospholipids

PLA$_2$: Phospholipase A$_2$

PLC: Phospholipase C

PMA: Phorbol myristate acetate

PS: Phosphatidylserine

PSN: prescalpular lymph nodes
PUFA: Polyunsaturated fatty acids
PWM: pokeweed mitogen
QR: Quantum red
SQ: Subcutaneous
RBC: Red blood cells
SFA: Saturated fatty acids
SSC: Side scatter
TBSA: Total body surface area
TC: T cytotoxic
TG: Triglyceride
TGF: Transforming growth factor
TH: T helper
TNF: Tumor necrosis factor
TPN: Total parenteral nutrition
UV: Ultraviolet

I.

Lipids, Immunity and Disease

A. Dietary Fat and Lipid Biochemistry

Triglycerides (TG), phospholipids (PL) and sterols provide the lipid component of the typical Western diet with TG accounting for approximately 95% of fat intake (Groff et al.,1995). Lipids ingested in these forms are hydrolyzed by pancreatic lipase and absorbed in the form of monoglycerides. The gastrointestinal mucosa reassembles the monoglycerides into TG and packages them into chylomicrons which are carried throughout the body and metabolized by the liver and peripheral tissues. Lipids cannot be directly transported in the blood, although a small amount may be esterified to albumin, therefore, the liver assembles exogenous fats it receives from dietary sources together with endogenous fat sources into very-low and low-density lipoproteins and secretes them into the blood. Fatty acids are then made available to the tissues by the action of lipoprotein lipase where they can be used for energy or *de novo* synthesis of fatty acids. Acylation to a polar head group allows for phospholipid (PL) formation and incorporation into biological membranes (Kinsella,1990).

Our diet provides us with a wide variety of fatty acids including saturated (SFA, palmitic, stearic, myristic), monounsaturated (oleic) and polyunsaturated (PUFA, linoleic and linolenic) and a small amount of long chain PUFA. Since 1929 it has been established that linoleic (18:2n-6) and linolenic (18:3n-3) acid are essential in the diet of humans (Burr et al.,1930). Our bodies can metabolize these basic fatty acids through elongase and desaturase pathways into specific fatty acids needed by our body (Figure I.1). A deficiency in essential fatty acids results in a manifestation of several clinical symptoms as well as lymphoid atrophy, decreased antibody responses and increased susceptibility to infections (Gurr,1992; Linscheer et al.,1988). Essential fatty acids are key structural components in membranes and are the only source of prostanoids (Gerster,1995). The ratio of n-6 to n-3 fatty acids is an important dietary consideration as there is competition for metabolic enzymes and tissue incorporation between these 2 families. It is recommended that the ratio of n-6 to n-3 fatty acids in the diet should be between 4:1 and 10:1 (Groff et al., 1995).

Linoleic acid comprises the majority of n-6 fatty acids in the diet and is readily obtained from corn, sunflower, soybean and safflower oils. 18:2n-6 is necessary for physiological function of many cellular systems. Arachidonic acid (20:4n-6), which is the most common fatty acid found in membrane PL (Kinsella,1990) can be obtained through the diet or derived from 18:2n-6 (Figure I.1). 20:4n-6 gives rise to the 2 and 4 series of prostanoids and leukotrienes which have immunosuppressive and inflammatory properties (Figure I.2).

Linolenic acid is another dietary essential fatty acid found in soybean, canola, and linseed oil as well as green plant tissues. Linolenic acid is metabolized to EPA (20:5n-3) and DHA (22:6n-3; Figure I.1) for incorporation into membranes. The conversion of linolenic acid to EPA and DHA is reported to be less in humans (Dyerberg et al.,1980), particularly in times of stress (Brenner,1981), therefore, sources containing EPA and DHA would guarantee their appearance in cell membranes and tissues. Fish oil is rich in long chain n-3 PUFA. The 3-series prostanoids and 4-series leukotrienes are formed from n-3 fatty acids. These are less immunosuppressive and inflammatory than the 2-series and 4-series produced from 18:2n-6 (Figure I.2). EPA is readily incorporated into membranes (Croset et al.,1992) and displaces some of the 20:4n-6 (Weaver et al.,1985). When high amounts of linolenic acid are consumed, there is inhibition of linoleic and 20:4n-6 metabolism (Corey et al.,1983; Lokesh et al.,1988; Yoshino et al.,1987). Therefore diets rich in n-3 fatty acids will decrease the amount of cyclooxygenase products derived from 20:4n-6 (Corey et al., 1983; Dyerberg et al.,1978; Needleman et al.,1979).

The dietary requirements for specific fatty acids during critical illness are not known, and this issue is heavily debated. Alterations in fatty acid metabolism and increased use of specific fatty acids by various tissues make the requirement different than that of healthy individuals. In addition, the ability of patients with burns or cancer to utilize specific fatty acids from the diet has not been determined. Metabolic pathways for *de novo* synthesis of fatty acids may be impaired during stress states and supplementation of certain fatty acids in the diet may be required.

B. Membranes: An Overview

A key role of lipids is to serve structural, barrier, antigenic, enzymatic and signaling functions within biological membranes. Cell membranes are composed of phospholipids made up of fatty acyl groups attached to a polar head group organized to form a lipid bilayer spanned with integral and peripheral proteins throughout (Figure I.3). The fluid mosaic model of Singer and Nicholson describes the basic general structure of membranes (Singer et al.,1972). Continuous, fluid barriers between the exterior and interior of cells and its various organelles compartmentalizes functional cellular components. Highly specific lipid/protein and lipid/lipid domains give rise to structural and functional characteristics of membranes (Clandinin et al.,1991). There may be a requirement of different proteins for particular phospholipids and phospholipids can be comprised of different fatty acids with varying physical properties (Kinsella,1990) exemplifying the complexities of membrane formation. Membranes are comprised of different percentages of proteins (25-70%), lipids (20-80%) and carbohydrate depending on the biological membrane of interest (Kinsella,1990).

Membranes provide working environments for a wide range of enzyme and hormone receptors and antigens that function at the aqueous lipid interface. Different membrane proteins are affected differently depending on their position in the fatty acid membrane in which they exist (Kinsella,1990). There are several examples of proteins that require specific PL or fatty acids for optimal function (Field et al.,1989; Kinsella,1990; Yeagle,1989).

Fluidity is a biophysical concept used to describe the resistance to movement of various types of molecules within membranes (Peck,1994a) and is an important factor in determining cell function. Fluidity of a membrane is altered by diet and disease. Fluidity is most influenced by the amount of unsaturation in the phospholipid (Hagve,1988). A higher polyunsaturated to saturated fatty acid (P/S) ratio in the membrane increases membrane fluidity (Berlin et al.,1989; Leger et al.,1990) which is usually associated with improved functional activities (Peck,1994b).

Studies on the effects of dietary fat intake on fat deposition show a relationship between what is consumed and appearance in tissues (Field et al.,1985). Likewise, changing lipid sources in one's diet causes compositional alterations in cellular phospholipids (Chapkin et al.,1990; Clamp et al.,1997; Clandinin et al.,1985; Tiwary et al.,1987). Fatty acids from dietary lipids are incorporated into different PL within the plasma membrane and are clearly altered by the dietary availability of fatty acids, although the responsiveness to dietary changes varies among the different PL classes (Clandinin et al., 1991). Therefore, dietary fat intake is reflected in membranes and impacts on cellular function. The mechanisms by which dietary fat influences function of immune cells specifically will be discussed in Section I.F.

Although severe injury and sepsis result in decreased fluidity (Franceschi et al.,1989; Todd et al.,1991), burn injury has been reported to increase fluidity of peripheral blood cells (Tolentino et al.,1991). Changes in fluidity occurring as a direct result of alterations in the composition of fatty acids in membranes after burns and during cancer and its treatment have not yet been established. The extent to which dietary lipids are reflected in membranes of immune cells and the subsequent effect this has on their function during these disease states has not been investigated. Given that cellular function is affected by the composition of PL in the membrane, and that dietary fat can change the membrane fatty acid profile, it is necessary to investigate the changes in fatty acid composition in immune cell membranes of patients in stress states and how this impacts on biological processes.

C. Metabolism

1. Macronutrient Metabolism During Stress

To better understand the complexities of feeding patients during states of stress, an understanding of metabolic changes that occur during the stress response is necessary. There are many differences in the metabolism of critically ill patients versus healthy fasting individuals (Figure I.4; Groff et al., 1995). During starvation there is a specific adaptive response that is designed to preserve lean body mass. During the stress response, however, hormones, cytokines and inflammatory mediators complicate and heavily influence the way in which fatty acids are metabolized. Energy substrates are mobilized to support inflammation, repair of tissues and maintenance of immune function at the expense of lean body tissues.

a) *Burns*

Burns induce hypermetabolism which is characterized by nutritional depletion, protein catabolism and immunosuppression beginning within 48 hours of trauma and reaching its peak between 7 to 10 days after injury. Burn trauma induces greater amounts of hypermetabolism than any other type of injury and patients have been reported to double their normal metabolic rate (Long et al.,1976; Shaw et al.,1987b; Tredget et al.,1992). Adequate nutrients are necessary during the stress response and are essential for prevention of malnutrition and associated complications, infection and mortality (Barton,1994).

(i) Protein Metabolism

Unlike starvation, which preserves lean body mass by utilizing alternate fuels, stress hypermetabolism can cause increases in protein catabolism to 2/3 above normal (Ireton-Jones et al.,1991) resulting in significant nitrogen losses (Barton,1994). Up to 1000 g of muscle mass is lost per day (Shaw et al.,1989) resulting in a critical reduction in lean body tissue within 14 days versus 60-90 days in fasting individuals (Cooper,1997). The reason for this catabolic response is due to the body's increased demand for glucose. The wound, brain, blood and bone marrow are obligate glucose users (Van Way,1991). Glucose is derived from amino acids which are obtained from protein reserves in muscle. Synthesis of metabolically important acute phase proteins and those used by the immune system are dependent upon protein degradation as well. Strategies to decrease protein catabolism by pushing the utilization of other energy substrates have been largely unsuccessful in diminishing the metabolic stress response (Cerra et al.,1980; Long et al.,1977; Shaw et al.,1987a). Protein catabolism for use as a gluconeogenic substrate occurs even when other energy sources, such as fat, are provided in excess (Streat et al.,1987), demonstrating an inability of exogenous nutrients to affect existing substrate oxidation patterns. Early enteral feeding has been shown to minimize but not reverse the hypermetabolic response (Alexander,1980; Moore et al.,1986; Moore et al.,1991). Nutritional therapy is necessary to maintain nutritional status, promote protein synthesis and provide energy. The amount of protein or types of amino acids that should be included a nutrient formulation is debated and varies with different disease states. A highly stressed patient may need a nonprotein calorie to nitrogen ratio of 80:1

or 100:1 compared to the normal recommendation of 150:1 (Barton,1994; Ireton-Jones et al., 1991). Maximal protein synthesis has been reported to occur when provided at 1.5-2.5 gm/kg body weight/d (Wolfe et al.,1989).

(ii) Carbohydrate Metabolism

Carbohydrates are the main source of energy for critically ill patients (Barton,1994). 60-70% of nonprotein calories have been recommended to come from glucose during septic states (Barton,1994). Observations of elevated glucose metabolism including turnover, clearance and oxidation have been supported by stable isotope data (Shangraw et al.,1989; Wolfe et al.,1987). Hyperglycaemia results from increased gluconeogenesis, glycogenolysis, insulin resistance and Cori cycle activity (Black et al.,1982; Jahoor et al.,1986). Glycerol and amino acids are major gluconeogenic substrates (Shaw et al.,1985). Infusion of glucose, however, does not suppress gluconeogenesis from amino acids (Cerra,1987). High amounts of glucose can exacerbate the hyperglycemia that occurs and result in fat deposition (Ireton-Jones et al.,1987). Provision of insulin has been shown to be rather ineffective because glucose oxidation is already maximal and endogenous insulin secretion is high (Barton,1994). Furthermore, there is an impaired ability to oxidize glucose (Shaw et al., 1987b; Wolfe et al.,1986). Providing excessive amounts of carbohydrate can potentially increase oxygen consumption, carbon dioxide production and fat synthesis (Ireton-Jones et al., 1987). Studies have shown that provision of excess glucose may be converted to fat due to an inability to oxidize more than 5 to 7 mg/kg (Sheridan et al.,1998; Tredget et al., 1992; Wolfe et al., 1986). Adding lipids to a glucose/amino acid mixture has been shown to decrease endogenous glucose production and improve nitrogen balance (Demichele et al.,1989). When fat is provided, it is used by tissues that do not rely solely on glucose for energy and there is less need to oxidize protein precursors for glucose synthesis (Demichele et al., 1989).

(iii) Fat Metabolism

Following burn injury, there are increases in fatty acid oxidation, free fatty acid turnover and lipolysis. Hypertriglyceridemia is characteristic of septic patients and is due to increased mobilization from stores, decreased clearance, increased synthesis and/or release from the liver (Gallin et al.,1969). Studies have shown fatty acids to undergo futile cycling through resynthesis of TG both intracellularly and between organs (Tredget et al., 1992; Wolfe,1996). Therefore, energy from this concentrated energy source is reduced and may contribute to overall increases in the metabolic rate (Shaw et al., 1987a). Hyperinsulinemia, resulting from elevated glucose concentrations in the blood of trauma patients, complicates lipid mobilization from fat stores. Isotope labelling of substrates have been used extensively to study the metabolism of glucose, protein and fat in severely traumatized patients (Shaw et al., 1987a; Wolfe et al., 1987). These studies have demonstrated increases in the rate of lipolysis and twice the basal fat oxidation rates of healthy volunteers which is consistent with other reports (Barton,1994).

In healthy humans, oleic acid is preferentially oxidized over linoleic and stearic acid (Jones et al.,1985). Prostaglandins derived from 20:4n-6 have been reported to stimulate lipolysis (Richelsen,1992). Lower plasma concentrations of 18:2n-6 and γ-linolenic acid (18:3n-6) have been observed in plasma fatty acids of burned compared to healthy rats (Karlstad et al.,1993). Post burn studies in humans have reported reduced plasma levels of 18:2n-6 and 20:4n-6 (Harris et al.,1981) with increased stearic and oleic acid (Cetinkale et al.,1997), profiles observed during states of essential fatty acid deficiencies (Barton,1994; Gottschlich et al.,1987) and consistent with increased lipolysis (Cetinkale et al., 1997; Smith et al.,1983).

The liver and GI tract are important organs governing absorption, synthesis and distribution of fat. Deficiencies in intestinal lipases (Carter et al.,1994) and the common occurrence of ileus following severe burn injury (Montegut et al.,1993) would impair the absorption of dietary fat. Free fatty acids are re-esterified at an accelerated rate in the liver rather than being oxidized for energy (Tredget et al., 1992; Wolfe et al.1987). The liver is the primary organ responsible for packaging of lipids into transport carriers. The liver is also responsible for the synthesis of acute phase proteins which would become a priority following burn injury. A transient deficiency in carnitine, an essential nutrient in fatty acid oxidation, has been reported post burn (Harris et al.,1982) which would reduce oxidation of fatty acids and is consistent with post morbid reports of fatty liver (Aarsland et al.,1996; Mittendorfer et al.,1998). A reduction in liver enzymes (Birke et al.,1965), damage of mitochondrial functions (Huang et al.,1998) and other liver abnormalities (Czaja et al.,1975) have been reported in stressed states. Carrier proteins, Apo CII, CIII and A1, necessary for lipid transport have been reported to be significantly reduced following burn injury (Vega et al.,1988).

Dysfunctions reported in the gastrointestinal system and liver would suggest alterations in fatty acid incorporation into lipoproteins may occur following burn injury. Alterations in lipoprotein metabolism have been consistently reported post burn since the first observation by Birke and colleagues (Birke et al., 1965). Consistent reductions in plasma PL and cholesteryl esters with elevated TG levels have been reported which may impact on delivery of fatty acids to tissues. As with other cells in the blood, the incorporation of n-3 fatty acids into cells of the immune system occurs within approximately 2 weeks through a direct exchange with plasma (Gibney et al.,1993). The fatty acid composition of plasma plays a role in determining cellular composition of immune cells whose function are affected by fatty acid composition of membrane PL.

In order to better understand impairments in regulatory biochemical processes governing the metabolism of specific fatty acids following burn injury, an analysis of the composition of plasma components is required. Fatty acids in plasma represent those available from the diet, those released from tissues and represent those available to tissues. These investigations will aid in understanding alterations in fatty acid absorption and metabolism and how these may be related to changes in fatty acid composition of immune cell membranes.

b) *Cancer and Cachexia*

Cancer cachexia is characterized by anorexia, weight loss, muscle loss, and abnormalities in macronutrient metabolism (Wigmore et al.,1997). Catabolism associated with the malignant disease process and all forms of anti-neoplastic therapies contribute to the development of cachexia in cancer patients (Torosian,1995). Malnutrition and weight loss significantly reduce survival following cancer treatment (DeWys et al.,1980; Ovesen et al.,1993a). Impaired wound healing, organ dysfunction and reduced immune function lead to post-operative complications and sepsis. Cachexia perpetuates the malnourished state and the efficacy of nutritional intervention in stopping this response has not been demonstrated (Ovesen et al.,1993b). Anorexia accompanies cachexia and nutrients that are consumed are not metabolized normally. This minimizes the benefits of dietary supplementation aimed at improving nutritional status in these patients.

Not all cancer patients are hypermetabolic as metabolic rates have been reported to be increased, decreased or unchanged depending on the characteristics of the tumour and the host (Giacosa et al.,1996). In addition, not all cancer patients are malnourished. The approximate intake of malnourished cancer patients is 1200 kcal (Bruera et al.,1984; Giacosa et al., 1996) compared to estimates of greater than 2000 kcal in cancer patients with normal nutritive status. Malnourished cancer patients have alterations in metabolism and body composition. Cachexia causes increases in protein degradation, reduced synthesis of proteins and increased gluconeogensis. Muscle mass is used as the first source of amino acids followed by visceral and circular proteins such as albumin (Giacosa et al., 1996). Body cell mass and body fat are both decreased (Shike et al.,1984; Shizgal,1985; Sukkar et al.,1993) and the extracellular mass is increased in severe cases (Sukkar et al., 1993). Fat mobilization from adipose tissues (Kitada et al.,1982; Taylor et al.,1992) and oxidation of free fatty acids are increased (Douglas et al.,1990; Hansell et al.,1986). Patients have been reported to be hyperlipidemic and this is associated with decreased lipoprotein lipase activity and/or increased hepatic lipid secretion (Memon et al.,1992).

Differences in essential fatty acids in the plasma of cancer patients compared to healthy individuals or those with benign disease have been reported. In patients with prostatic cancer, plasma PL have been reported to contain significantly greater amounts of 18:1n-9 (Chaudry et al.,1991) and reduced levels of 18:2n-6 and 20:4n-6 (McClinton et al.,1991) compared to healthy individuals. In patients with bladder cancer, 18:2n-6, 20:4n-6, EPA and DHA in plasma were reported to be lower than in controls (McClinton et al., 1991). These plasma profiles are consistent with deficiencies in essential fatty acids.

During anorexia/cachexia, nutrients normally needed for growth and maintenance of muscle mass are shunted towards the immune system (Kern,1988) and production of acute phase proteins. Proteins synthesized to support the acute phase response have been used as strong indicators of poor prognosis in cachexic cancer patients (Falconer et al.,1995). Attempts to ameliorate the cachexic condition through provision of nutrients have been unsuccessful in the past. However, recently, it was demonstrated that the essential fatty acids, EPA and DHA, can decrease the production of inflammatory cytokines thought to be involved in the etiology of cachexia, giving promise to the notion that dietary fat may be able to reduce the cachexic response.

In patients with advanced pancreatic cancer, which induces the most rapid weight loss of all cancer types, supplemental n-3 fatty acids in the form of fish oil have been shown to prevent further weight loss and promote weight gain in some patients (Wigmore et al., 1996; Barber et al.,1999; Wigmore et al.,1997) The mechanisms for these effects have not been entirely elucidated, however, reduced production of catabolic cytokines, reduced tumour growth, stimulation of anti-cancer immune defenses of the host, and an attenuation of the acute phase response (APR) have been proposed. Dietary fish oils have been shown to reduce the APR and release of pro-inflammatory cytokines *in vitro*, in animals and in healthy humans (Endres et al.,1989; Falconer et al.,1994; Meydani et al.,1991). Dietary fish oils have also been shown to attentuate serum C3 factors and reduce the APR in pancreatic cancer patients (Wigmore et al., 1996; Wigmore et al., 1997) possibly by reducing IL-6 production (Castell et al.,1990; Heinrich et al.,1990).

N-3 fatty acids can act on host anti-tumour mechanisms or the tumour to reduce cachexic responses. The majority of tumour cell lines representing a variety of animal and human cell types have been shown to respond to fatty acids of the n-6 and n-3 series through slowing of cell growth (Beck et al.,1991; Falconer et al., 1994) or induction of apoptosis (Horrobin,1992; Jiang et al.,1998). Feeding n-3 fatty acids have been shown to reduce tumour incidence in animals (Beck et al., 1991). Reduced tumour growth could be an effect of fish oils on the tumour directly or a stimulation of host immune function to reduce tumour size. However, it is unlikely that reduced tumour growth in patients with advanced cancer or widespread metastastis would impact on weight loss so dramatically within a relatively short period of time.

The ability of cachexic patients to absorb and metabolize n-3 fatty acids is not well characterized. Whether these fatty acids get incorporated into host tissues or cells of the immune system and what dose is required to see these effects is not known. Studies have shown cancer patients to exhibit plasma and erythrocyte profiles characteristic of essential fatty deficiencies. To what extent cachexic cancer patients are able to utilize dietary fatty acids to overcome reduced essential fatty acid status with n-3 fatty acid supplementation is not known. Given the beneficial effects of fish oil on reducing the cachexic response, it will be important to determine the appearance of these fatty acids in tissues such as those of the immune system where factors contributing to the etiology of cachexia are thought to be derived.

c) *Mediators in the Metabolic Response to Stress*

The immune and acute phase responses of the host designed to fight infection and cancer are thought to be partially responsible for perpetuating the metabolic stress response. The endocrine balance is upset with insulin being balanced by increases in glucagon, cortisol and the catcholamines (Bessey et al.,1984; Stoner,1970). While the latter serve to mobilize energy stores, insulin is required for synthesis of proteins and storage of energy substrates.

Key cytokines thought to play a role in the metabolic stress response are IL-6, TNF-α and IL-1. An overview of their effects on various tissues is shown in Figure I.5. Elevated plasma levels of IL-1β, IL-6 and TNF-α, have been reported in sepsis (Blackwell et al.,1996), surgical trauma (Blackburn,1992; Szabo et al.,1991), burn injury (Drost et al.,1993; Pascal de Bandt et al.,1994) and cancer (Tisdale,1999). The combined effects of these cytokines are greater than a single one alone (Last-Barnet et al.,1988). Blocking or neutralizing these cytokines has been shown to reduce the cachectic response and improve survival in animal models (Souba,1994).

IL-1 and TNF-α, produced largely by activated macrophages, exert synergistic effects on fat metabolism (Grunfeld et al.,1991; Hardardottir et al.,1992b). Administration of IL-1 and TNF-α *in vivo* elevates TG and free fatty acid levels. They also suppress lipoprotein lipase activity (Freid et al.,1989; Hardardottir et al.,1992a; Hauner et al.,1995; Zechner et al.,1988) which serves to deplete fat stores (Freid et al., 1989; Freid et al.,1993). Adipocytes have receptors for TNF-α (Kern,1988) and it is likely that these cytokines play a role in lipid metabolism perturbations that occur during stress.

IL-1 and TNF-α act synergistically on production of 20:4n-6 metabolites (Elias et al.,1987). 20:4n-6 is liberated from phospholipids upon stimulation of synovial cells with IL-1 (Angel et al.,1993). Infusion of TNF-α into animals has been shown to result in a loss of n-6 fatty acids from tissues, particularly 18:2n-6 and 20:4n-6 (Raina et al.,1995). In healthy humans, 20:4n-6 metabolites such as PGE_2, feed back on the production of inflammatory cytokines (Roper et al.,1994). Burn injury, however, has been reported to result in a loss of down-regulation of TNF-α synthesis induced by PGE_2 (Molloy et al.,1993; Peck,1999). The effects of prostaglandins on tissues may be partially due to their inhibitory effects on pro-inflammatory cytokines as their effects oppose those of IL-1, TNF-α and IL-6. One mechanism by which PGE_2 works is through inhibiting adenylate cyclase. Decreased cAMP concentrations (Lambert et al.,1980) have been shown to reduce gluconeogenesis and glycogenolysis *in vitro* (Brass et al.,1984; Brass et al.,1985; Imesch et al.,1975) while accelerating lipid synthesis and decreasing lipolysis (Lambert et al., 1980; Richelsen et al.,1985). Other studies have shown prostaglandins to have a role in glucose oxidation and glycolysis (Czech et al.,1972; Leighton et al.,1985).

Although IL-1 and TNF-α have been thoroughly investigated, conclusions regarding levels of these cytokines post burn are inconclusive. No change (Chen et al.,1997; Ogle et al.,1994; Pejnovic et al.,1995), increases (Drost et al., 1993; Grayson et al.,1993; Wu et al.,1995) or a lack of correlation with burn size (Drost et al., 1993) have been reported. Variations in these studies may be due to the timing of measurements following injury, the tissue of study or the methodologies used. More consistently, however, increased IL-6 levels have been reported in animals and humans following burn and has been found in wound exudates (Grayson et al., 1993; Pejnovic et al., 1995), mucosal associated tissues and cells (Ogle et al., 1994; Rodriquez et al.,1993) and plasma (Drost et al., 1993). IL-6 is associated with wasting of muscle and is known to be an important regulator of the acute phase response (Scott et al.,1996). IL-6 and TNF-α are higher in infected compared to noninfected burn patients and have been shown to relate to mortality during infection (Peck,1999).

TNF-α in the serum of cancer patients reflects severity of disease and relates to tumour size, presence of metastasis and extent of lymph node involvement but has not been shown to relate to the presence of cachexia (Tisdale,1999). Conversely, IL-6 is found to relate to weight loss in cancer (Scott et al., 1996). TNF-α and IL-6 have been shown to stimulate the ATP-ubiquitin proteolysis system (Llovera et al.,1997; Tsujinaka et al.,1996) and may contribute to muscle wasting through this mechanism. Other factors besides cytokines have been shown to be involved in cachexia (Beck et al.,1987; Todorov et al.,1996). Tumours have also been shown to produce catabolic products that stimulate both proteolysis and lipolysis (Todorov et al., 1996). A cachexic factor that serves to mobilize lipids and induce proteolysis has been isolated from cachexic cancer patients (Tisdale,1996) but not from patients who have not lost weight nor in other diseases characterized by hypermetabolism such as burns. This proteolytic/lipolytic factor is thought to be an intermediate in lipid metabolism. Inhibitors of the lipoxygenase pathway have also been reported to inhibit tumour growth and cachexia (Hussey et al.,1996).

It is known that mediators of the stress response have profound consequences on fatty acid metabolism. Supplementing the diet with n-3 fatty acids, at least in cachexic cancer patients, has been shown to overcome many complications of the wasting response. The extent to which essential fatty acid metabolism is altered in stress states such as burns and cancer and the ability of this response to be improved with supplementation of fatty acids in the diet is not known. In order to better understand how mediators of the stress response impact on essential fatty acid metabolism, an analysis of the fatty acids available to tissues as well as their incorporation into immune cells membranes is required.

D. The Immune System

1. The Innate Immune System

The major components of the innate immune system are shown in Figure I.6 and will be discussed in more detail below.

a) *Physical barriers*

Skin and mucous membranes provide mechanical barriers and offer the first line of protection against invading microorganisms. Skin contains keratinocytes and Langerhans cells that serve as antigen presenting cells (APCs) (Nickoloff et al.,1995). Keratinocytes contain large amounts of IL-1 which is released upon insult (Boehm et al.,1995; Matsue et al.,1992). Immune cells that reside in mucosal associated lymphoid tissues play important roles in the protection against potentially infectious agents introduced at mucosal surfaces (Brandtzaeg et al.,1989). A vast immune system also exists in the gastrointestinal tract that prevents microbial, toxic or antigenic antigens from entering the body (Cunningham-Rundles et al.,1998). Immune cells are located in intraepithelial regions of the gut and specialized lymph tissues known as Peyer's Patches. The main defense in the gut comes from the large number of activated B cells present in the lamina propria region that secrete IgA (Cunningham-Rundles et al., 1998). The complex interactions between B cells, T cells, macrophages and mast cells of the gastrointestinal tract protects the host from infectious agents introduced at this site.

When physical barriers are penetrated by infectious organisms, the cellular components of the innate immune system respond immediately and activate the acquired/learned immune system. The innate (non-specific) immune system is comprised of defenses that are present early in life and function without depending on previous exposure to a particular pathogen. This arm of the immune system provides the initial host defense, protecting the individual until the acquired (specific) immune system becomes activated.

b) *Macrophages*

Monocytes leave circulation to enter extravascular tissues where they differentiate into macrophages. Killing of bacteria by macrophages involves phagocytosis and generation of reactive oxygen species. They are essential not only in directly destroying organisms but also in processing and presenting antigen to T-helper cells to initiate the second line of defense. The production of interleukin-1 (IL-1) stimulates T helper cells to produce interleukin-2 (IL-2), which activates and expands both T and B lymphocyte populations (Alam,1998). Activated macrophages stimulate acute inflammation processes through production of TNF-α and IFN-γ and are also the main producers of PGE_2. Mediators produced by macrophages will be discussed further in Section I.D.3.

c) *Natural Killer Cells*

Natural killer (NK) cells are considered to be a subset of lymphocytes found in blood and lymphoid tissues. NK cells are non-MHC dependent cells that recognize and destroy cells that have become transformed by viruses (Levitz et al.,1993; Murphy et al.,1993). They have also been shown to have direct toxic effects on infected cells. Killing of targets by NK cells is similar to that of the cytotoxic T cell and effector functions include granule exocytosis, release of pore-forming proteins and apoptosis (Trinchieri,1998). NK cell functions are influenced by many factors such as cytokines, eicosanoids and immunoglobulin complexes (Jondal,1987). NK cells have been shown to have increased cytotoxicity when treated with IFN-γ, IL-12, TNF-α or IL-2 *in vitro* (Bancroft,1993; Markovic et al.,1993; Singh et al.,1992).

d) *Neutrophils*

Neutrophils are an essential component of innate immunity and provide the first line of defense against invading pathogens. There are several steps important in the activation of neutrophils that ensure an optimal response capable of clearing infectious agents. When tissue is injured, mediators such as complement cascade, fibronectin, vasoactive amines and chemoattractants are released. Adhesion molecules present on the neutrophil membrane allow them to migrate towards chemoattractants, adhere to and transmigrate across the microvascular endothelium to accumulate at sites of tissue injury (Brom et al.,1995; Damtew et al.,1993). Neutrophils then detect, ingest and destroy bacteria and other foreign particles that have invaded the mechanical barriers. They have 2 basic mechanisms by which to do this. The first involves the nonoxidative killing system which utilizes degradative enzymes, such as lysozyme, to destroy ingested bacteria (Benhaim et al.,1992). The capacity of phagocytes to generate reactive oxygen intermediates is also an essential part of the human defense mechanism against infectious agents. The phagocytosis process activates enzymes that produce superoxide from atmospheric oxygen which is toxic to invading pathogens (Figure I.7).

Neutrophil activation is mediated by cytokines, such as IL-1, and TNF-α, which stimulate oxidative burst and granule release (Fujishima et al.,1995) as well as IL-8 which in addition to the above functions also acts as a chemoattractant. Lipid derived factors such as eicosanoids, lipoxin and platelet activating factor are also important in stimulating neutrophil function (Fujishima et al., 1995). Circulating factors can modulate neutrophils into a state of enhanced responsiveness towards a secondary stimulus (Konig et al.,1992).

The multicomponent NADPH oxidase enzyme generates oxidative compounds toxic against bacteria. The importance of this enzyme becomes apparent in patients with chronic granulatomous disease who have recurrent life threatening infections (Gallin et al.,1980). When a neutrophil becomes activated, the membrane and cytosolic components assemble. The reaction catalyzed by the NADPH oxidase is shown in Figure

I.7. This response can be quantified by the use of dihydrorhodamine, a freely permeable dye that localizes in the mitochondria. Upon activation of the NADPH oxidase enzyme and subsequent production of oxidative compounds, the dye is reduced to a colour that emits a highly fluorescent signal upon excitation by blue light (488nm) assessed by flow cytometry (Figure I.8). It is highly specific for respiratory burst activity (Rothe et al.,1994) and one is able to classify neutrophils of different oxidative capacities using this method.

2. The Acquired Immune System

Many different immune cell types comprise the acquired immune system, as illustrated in Figure I.9. The T cell population is divided into 2 basic subsets CD4 (helper/inducer) and CD8 (cytotoxic/suppressor) cells. T-lymphocytes serve many functions including providing help to B cells, stimulating the inflammatory response, maintaining antigen-specific memory and producing cytokines (Alam,1998). Cell mediated immunity is important for the elimination of intracellular bacteria, virally infected or tumour cells and foreign grafts either through direct cytotoxic killing ability or through the recruitment of other cells that have the capacity to eliminate the foreign antigen. Humoral immunity, mediated by B cells, is important in the immune response to extracellular pathogens, parasites and allergens (Abbas et al.,1994). The interactions between cells of the acquired immune system are modulated by a number of mediators, including cytokines which will be discussed in further detail Section I.D.F.

a) *T-Lymphocytes*

CD4 cells are central to the mounting of both humoral and cell mediated immune responses. In response to an infection, lymphocytes must be able to proliferate and expand to produce enough cells to combat invading organisms. This process begins with the interaction of the T cell receptor with an antigenic peptide presented in association with MHC II on the surface of an antigen presenting cell. Antigen presenting cells can be macrophages, B-cells or dendritic cells. Proliferation of T cells occurs in response to recognition of antigen through secretion of its own growth promoting cytokines and corresponding cell surface receptors (Abbas et al., 1994). Cells that are responsive to a specific antigen can then develop into antigen-specific memory cells which are capable of initiating larger secondary responses upon subsequent exposure to the antigen. Release of cytokines by the macrophage and the T cell recruits other required immune cells to the site. Cytokines secreted by T cells can act on other T cells, B cells, macrophages or the endothelium. CD4 cells can be further classified as TH1 or TH2 depending on the cytokine pattern they secrete (Mosmann, 1996).

Cytotoxic T-cells (CD8) can directly kill infected cells through recognition of antigen in association with MHC class I present on every cell in the human body. These T cells contain granules that contain perforin, a substance that creates "pores" on the target cell membrane and allows for the entry of lytic substances into the cell (Abbas et al., 1994). Destruction of infected cells can also be carried out through apoptosis thought to be mediated by cytokines secreted by the cytotoxic T cell. Lysis of a target requires cell contact and the lytic mechanisms are directed only toward the infected cell. CD8 cells also produce cytokines such as IFN-γ to stimulate cells of innate system such as macrophages.

The principal autocrine growth factor for T-lymphocytes is IL-2 and CD25 is identical to its high affinity receptor. CD25 is commonly used as a T cell activation marker. The presence of IL-2 is sufficient and necessary for the expansion of most T and B-cells and NK cells. IL-2 is produced by most T cells during the early stages of the immune response and facilitates proliferation and expansion of most lymphocyte subsets (Swain,1991). Lipoxygenation of 20:4n-6 is stimulated in the target cell and mediates intracellular signal transduction to regulate secretory activity and/or cellular proliferation (Farrar et al.,1985). CD71 or the transferrin receptor is another marker of T cell activation. Its expression usually preceeds that of CD25.

b) *B cells*

The humoral immune system is made up of B cells capable of producing and secreting antibodies for the elimination of extracellular pathogens. B cells can also serve as antigen presenting cells and are most abundant in lymph nodes. In order to become activated into antibody secreting plasma cells, they must present antigen to T-helper cells which, through cytokine release, directs the B cell into producing specific antibodies that bind to the foreign particle and facilitate phagocytosis by cells of the innate system (Alam,1998; Fleisher,1997; Goust et al.,1993; Parker,1993).

Cell mediated, humoral and innate branches work together to eliminate intracellular and extracellular pathogens (Seder et al.,1999). A schematic of how the cellular components work together to mount an immune response is shown in Figure I.10. Appropriate interactions between the different cells of the immune system are necessary in order to carry out the effector functions necessary for elimination of a particular antigen and are orchestrated by cytokines (Discussed in Section I.D.3). The most appropriate immune responses that must be expanded to eliminate a specific type of infection are shown in Table I.1.

c) *Leukocyte Common Antigen (CD45)*

The CD45 molecule, or common leukocyte antigen, is found an all white blood cells. In addition to its phosphotyrosine activity, it also can be used as a differentiation marker. The isoforms of the leukocyte common antigen result from differential splicing of the exons from the gene encoding CD45 and are A, B, C and O (Kuschnaroff et al.,1997) with RA and RC representing naive cells and the O and B isoforms representing primed lymphocytes. The various isoforms of the common leukocyte antigen are not mutually exclusive and cells expressing both naive and mature markers are in a transitional state. RO expression increases during differentiation inversely to the disappearance of the RA antigen (Salmon et al.,1994). By examining cellular expression of the different forms of CD45 that exist, one can extrapolate recent activation events of individual T cells. Differential expression of CD45 isotypes is associated with memory function (Sanders1988). CD4/CD45RO+ cells are better able to proliferate than CD45RA+ cells and CD8/45RO+ cells contain the effector cytotoxic cells (Johannisson et al.,1995). A clear correlation between DNA synthesis and phenotype switching has been observed suggesting alterations from CD45RA to CD45RO accompanies proliferation (Johannisson et al., 1995).

d) *Flow Cytometry*

Flow cytometry is a technology that has been cornerstone in the explosion of immunology research in the past decade. This technology can measure characteristics of a single cell in suspension based on its light scatter and fluorescent properties. A laser light source measures the amount of light scattered in the forward and side (perpendicular to the direction that the light is travelling) direction and detects them using forward scatter (FSC) and side scatter (SSC) channels, respectively (Melamed,1990). FSC and SSC are proportional to the size, shape and homogeneity of the cells being measured. These characteristics can be used to distinguish live and dead cells as well as granular versus nongranular cells When fluorescent labelled monoclonal antibodies are used, or other fluorescence emitting compounds, fluorescence is detected in a unique fluorescence channel. Multiple detectors are used to simultaneously measure more than one scatter or fluorescence from a cell. In this manner, R-phycoerythrin (PE), fluoroscein isothiocyanate (FITC) and quantum red™ (QR) can be used to detect cell surface markers on a single cell.

3. Mediators of the Immune System

The effector responses of immune cells in both natural and specific immunity are mediated by cytokines, regulatory proteins released by immune cells in response to a variety of inducing stimuli. Upon release and binding to their target cell they induce changes in gene expression in the target cell. Cytokines are responsible for communication between cells of the immune system as well as other cells in the body and regulate the type of immune response that will occur when the host is faced with a specific type of infection. The localized concentration of various cytokines will determine what immune cells are activated.

Among the CD4 subset (discussed above) there is further subdivision into classes of immune cells based on the cytokines they produce and secrete. TH1 cells produce IL-2 and IFN-γ and generally promote a cell-mediated inflammatory response. TH2 cells produce IL-4, IL-5, IL-10 and IL-13 which support a humoral antibody response (Romagnani et al.,1995). In addition, TH0 cells have been described that express cytokine patterns characteristic of both TH1 and TH2 cells and TH3 cells which produce TGF-β. TGF-β, another growth stimulating cytokine, has highly variable functions depending on the cell type of interest (Letterio et al.,1998; Sosroseno et al.,1995). Although these patterns of cytokines are not as clearly defined in humans as they are in mice, it has been proposed that the balance between these two responses offers a mechanism by which the immune system can respond appropriately to a specific type of infection (Mosmann et al.,1996).

IL-1 is produced by macrophages and other cells of the monocytic, phagocytic lineage (Monge et al.,1991) and promotes the inflammatory response (Dinarello,1988) (Mizel,1989) by stimulating release of TNF-α and IL-6. The IL-1 produced by macrophages activates T cell proliferation whereas TNF-α is involved in cytotoxicity (Beutler,1988; Sherry et al.,1988). At low levels, TNF-α enhances immune defense whereas systemic release causes septic shock and tissue injury (Azzara et al.,1995). IL-1 and TNF-α work synergistically to induce shock by a process thought to involve eicosanoids (Dinarello et al.,1989). The role of these cytokines in the pathophysiology of inflammation and stress response was discussed in Section I.C.1.c. Since the production and function of cytokines are under the control of 20:4n-6 metabolism (Dinarello,1988; Knudsen et al.,1986; Okusawa et al.,1988; Rappaport et al.,1982; Rola-Pleszczynski,1985), interest has arisen in ways in which to modify diet to alter these responses.

There are several other important mediators of immune function. Metabolites of the lipoxygenase and cyclooxygenase pathways have potent immunosuppressive (Shapiro et al.,1993) or immunostimulatory (Liu et al.,1996) effects. Activated macrophages produce high amounts of PGE_2 which is thought to contribute to immunosuppression following trauma (Miller-Graziano et al.,1988). Leukotrienes, particularly LTB_4, are potent chemoattractants for neutrophils, enhancing their migration to the site of injury. Activation of the complement cascade has been demonstrated in several pathophysiological states (Bengtsson,1993; Davis et al.,1987; Heideman et al.,1984) and is important in mediating inflammation and promoting ingestion of microorganisms by phagocytes (Hebert et al.,1995). This pathway becomes activated during stress by antibody-antigen binding and IL-1. Complement peptides can also serve as chemoattractants to recruit phagocytes to an infected area (Benhaim et al., 1992) and opsonize bacterial antigens to facilitate phagocytosis. Nitric oxide is another mediator implicated in infection. It is important in bactericidal activity of macrophages (Jacob et al.,1993) and high levels may suppress neutrophil function. Catecholamines and glucocorticoids are released in response to stress and infection and have been shown to have immunosupppressive effects (Gennari et al.,1997).

4. Wound Healing

Healing of wounds is an important determinant in duration of recovery in both burn and cancer patients undergoing chemotherapy. Nutritional status and immune function are important contributors to the wound healing process. These factors will be discussed briefly.

a) *The Immune System and Wound Healing*

Wound healing is a dynamic process that follows an integrated, predictable pattern. It begins with the disruption of tissue homeostasis resulting in activation and release of messengers that provide signals for inflammation, fibroblast proliferation, and immune activation (Sheldon,1992). Factors contributing to wound healing can be intrinsic, such as the presence of infections, or extrinsic, such as nutritional factors, drugs or presence of disease (Lawrence,1992).

The effects of an impaired immune system and its impact on wound healing are evident in diseases like diabetes (Goodson et al.,1986; Morain et al.,1990; Rosenberg,1990) and advanced age (Gerstein et al.,1993; Holt et al.,1992). Immunosuppressive agents such as chemotherapy and steroid treatments delay wound healing activities (Cromack et al.,1993; Fishel et al.,1983; Leibovich et al.,1975; Mustoe et al.,1989). Manipulations that enhance immune function, such as arginine (Barbul et al.,1990), glutamine and n-3 fatty acids (Daly et al.,1988; Daly et al.,1992), also improve wound healing efficacy suggesting an integral role of immune system involvement in wound healing.

Several types of immune cells participate in the wound healing process, each serving a specific, integrated purpose in its orchestration. At each of the three stages of wound progression, different immune cells predominate (Figure I.11). Neutrophils are first to appear at the wound site, often within 6 hours and reaching peak numbers between 24 and 48 hours (Barbul et al.,1995). They engulf and destroy bacteria and other foreign materials that may have been introduced to the wound as a result of injury. Although wound healing can proceed normally in the absence of neutrophils (Witte et al.,1997), opsonization and killing by these cells is an absolute requirement for resistance to extracellular pathological agents (Alexander,1979). Macrophages are the second type of immune cell to infiltrate the wound and reach peak numbers at 3 days (Barbul et al., 1995). In addition to their phagocytic properties, they release growth factors and mediators important in the inititation and propagation of granulation tissue formation, proliferation and synthesis of fibroblasts as well as neovascularization (Barbul et al., 1995; Mutsaers et al.,1998). Unlike neutrophils, macrophages stay in the wound until it is healed. Macrophages, along with T lymphocytes, the third immune cell to appear, are critical to normal healing of the wound and release cytokines that stimulate progression of wound remodelling. T cell involvement in wound healing is clarified by studies that show decreased breaking strength, hydroxyproline and reparative collagen synthesis following a depletion of T cells *in vivo* (Barbul et al.,1989; Efron et al.,1990; Peterson et al.,1987). The balance of lymphocytes in the wound area, together with other types of cells important in wound healing, and the resulting pattern of cytokines produced, determines how the wound will progress. An imbalance of these factors, some of which are not yet known, may be what causes an inability of some wounds to heal normally.

b) *Nutrition and Wound Healing*

In addition to having an important role in the resistance to infection, nutrition plays a major role in the efficacy of wound healing. Malnutrition slows the healing process and causes inadequate or incomplete healing of wounds (Konstantinides et al.,1993). Nutritional adequacy or deficiency can impact at any of the 3 stages of wound healing. Energy needed for tissue maintenance and repair is provided by protein, fat and carbohydrates (Patten,1995). Carbohydrates provide most of the energy required for wound healing (George1996) and is necessary to spare protein stores (Patten,1995). Fats are needed for the synthesis of prostaglandins which mediate inflammation and cell metabolism (George et al.,1996). Essential fatty acid requirements are increased during tissue repair and a deficiency delays wound healing (Caffrey et al.,1981; Telfer et al.,1993). Construction of collagen and proteoglycans, as well as wound remodelling, vascularization and fibroblast proliferation are dependent on protein (Meyer et al.,1994). A protein deficiency can lead to decreased resistance to infection, limited phagocytic capabilities and impaired inflammatory responses (De-Souza et al.,1998).

E. The Immune Response During Stress

1. Burn Injury

a) *Innate Immunity*

(i) Macrophages

Macrophages are important regulators of other branches of immunity. They are the primary immune cells at the site of injury, therefore, abnormalities in their function precede and influence subsequent immune responses they generate. Hyperactivation of macrophages leading to dysregulation of PGE_2 and cytokine production contributing to the cascade of immune impairments following burn injury (O'Riordain et al.,1992). Following burn injury, elevated levels of IL-1 released by injured skin (Kupper,1989b) and macrophages, may result in supranormal levels of IL-1 in circulation or locally. High levels of IL-1 have been reported to reduce antigen presenting capacity of macrophages (Baker et al.,1987; Kupper,1989a) which would also impair T cell responses dependent on this interaction.

Early after burn, macrophages have decreased phagocytic ability, lysozymes, NADPH production and bactericidal capabilities (Griswold,1993). Changes that occur in the bone marrow after burn injury can enhance the ability of macrophages to become activated (Ogle et al.,1993). Oxidative metabolism of 20:4n-6 occurs upon activation of human monocytes and macrophages (Bruord, 1990) and PGE_2 is increased following burn injury (Fukushima et al.,1994; Grbic et al.,1991). PGE_2 has been found in the burn wound (Arturson et al.,1973; Heggers et al.,1980), surrounding lymphatic tissue (Angaard, 1970) and in serum (Arturson et al., 1973) of patients following burn injury. PGE_2 normally serves to downregulate induction of inflammatory cytokine production, such as IL-1 and TNF-α (Burch et al.,1989; Goodwin,1985; Kawakami et al.,1986; Kinsella et al.,1990b; Kunkel et al.,1986). However, increased amounts of PGE_2, along with increases in cytokines have also been reported (O'Riordain et al., 1992). Prostaglandins released by macrophages have been shown to depress several T cell functions including IL-2 production and expression of its receptor (Peck et al.,1991).

(ii) Neutrophils

Sepsis and multiple organ failure remain major obstacles in recovery from burn injury (Zidek et al.,1998). Neutrophils are pivotal in defense against infection and abnormalities in their function contribute significantly to development of infection in patients with burns (Alexander et al.,1978; Alexander,1979; Bjornson et al.,1989;

Damtew et al., 1993; Davis et al.,1980; Marino et al.,1988; McManus,1983). Every aspect of neutrophil killing has been reported to be affected by burn injury including chemotaxis (Bjornson et al.,1992; Davis et al.,1984; Davis et al., 1980; Deitch,1984; Moore et al., 1986; Nelson et al.,1987; Solomkin,1990), bactericidal activity (Alexander et al., 1978; Alexander,1979; Bjornson et al.,1986; Bjornson et al., 1989) and oxidative metabolism (Braquet et al.,1985; Dobke et al.,1989; Duque et al.,1985; Gadd et al.,1989; Heck et al.,1975; Heck et al.,1980) compromising early host defense against infection. Neutrophil dysfunction increases with severity of burn and depth of tissue injury.

Neutrophils present in circulation experience a sudden surge of activation due to the release of chemokines and injury mediators following burn injury. Chemotaxis, or migration, toward the injury is often slow and misdirected until open wounds are reduced to about 20% of open areas. Adhesion proteins are upregulated and neutrophils have been reported to adhere to the endothelium before they reach the injured site (Griswold,1993). Damage to host tissues can occur when oxidative radicals and degradative enzymes are released in these areas. Accumulation of neutrophils in the lungs is associated with ARDS (Fujishima et al., 1995). Pulmonary failure, manifested by ARDS and pneumonia, is currently the major cause of death following burn injury (Hansbrough et al.,1996). Primary or azurophilic granules containing hydrolytic enzymes, protease, lysozyme and myeloperoxidase, normally used to kill ingested organisms after phagocytosis, increase in activity. These histoxic secretions create a microenvironment between the neutrophil and endothelial tissue cell membrane where there is an accumulation of oxidants and proteolytic enzymes released by the neutrophil that surpasses antioxidant and antiproteinase mechanisms of the host, resulting in endothelial damage and degradation of the matrix (Wakefield et al.,1993). The rapid release of granules, followed by an inability to synthesize new granules, leads to an inability to kill phagocytosed organisms. Therefore the needed prolonged inflammatory response may be impaired due to this initial hyperactivation.

(iii) Natural Killer Cells and Burns

Burn injury has been reported to depress NK cytotoxicity (Blazar et al.,1986) which can last up to 50 days in severely injured patients. This depression may be attributed to the stress response following burn injury as infusion of hormones into healthy subjects reduces NK cytotoxicity (Blazar et al., 1986). Burn patients often undergo multiple surgeries which have been shown to reduce NK cytotoxicity (Pollack et al.,1991). Impairments in IL-2 production following burn injury may impact on the cytotoxicity of NK cells (Blazar et al., 1986; Singh et al., 1992).

b) *Acquired Immunity*

Several aspects of lymphocyte function are known to be reduced following burn injury. Burn injury consistently results in a depletion of circulating lymphocytes with a decrease in the helper (CD4) to suppressor (CD8) ratio, a measure of cell mediated immunity (Antonacci et al.,1984; Burleson et al.,1987; Hansbrough et al.,1989; McIrvine et al.,1982; O'Mahony et al.,1984; O'Mahony et al.,1985; Rodrick et al.,1983; Zapata-Sirvent et al.,1993). In an attempt to determine the distribution of lymphocytes following burn injury, Organ et al. (Organ et al.,1989) simultaneously examined immune organs at various timepoints following burn injury in rats. Decreases in lymphocyte numbers in all immune compartments was observed at post burn day 2 in rats which fails to demonstrate a sequestration or "lymphocyte trapping" of the immune cells from circulation to specific lymphoid regions. This does not, however, eliminate the possibility of a massive recruitment of the cells to the site of injury.

It is well established that both the production of IL-2 and its receptor are altered following burn injury (Sayeed,1996; Teodorczyk-Injeyan et al.,1986; Teodorczyk-Injeyan et al.,1987; Teodorczyk-Injeyan et al.,1990; Wood et al.,1984) and result in suppressed cell mediated responses. With the elucidation of the opposing patterns of cytokines secreted by T-helper cells, recent studies have demonstrated that rather than a suppression of helper activity *per se*, predominance of a TH2 cytokine profile results in immunosuppression. A TH1 response would be more effective in defenses against infections during this critical period. Evidence for a predominant TH2 resonse is given by increased production of IL-10 (Lyons et al.,1997) and IL-4 (O'Sullivan et al.,1997) in burn patients compared to controls, cytokines that inhibit TH1 responses (Kelly et al.,1997) and stimulate TH2 responses (O'Sullivan et al., 1997). Furthermore, mononuclear cells from burn patients have been shown to produce less IFN-γ and IL-2. Low levels of these cytokines are indicative of a TH2 response. Reduced cytokine production has been shown to be accompanied by reduced mRNA expression of the corresponding cytokine (O'Sullivan et al.,1995). These observations have been associated with septic events in humans (Lyons et al., 1997) and mortality in burned rats (O'Sullivan et al., 1995).

2. Infection

Attempts to reduce number of infectious incidences is a primary concern in the clinical setting. Infections can double length of hospital stay, increase the number of surgical procedures and result in immeasurable costs to patients such as delay of adjuvant therapies, and reduced quality of life (Bumpous et al.,1995). Host resistance to bacterial invasions is thought to primarily depend on non T cell branches of immunity such as acute phase protein production, complement, neutrophils and macrophages (Cantor et al.,1993). T cells produce cytokine and chemotactic factors that act on both innate and acquired immune functions to stimulate infection defense (Moran et al.,1988; Ras et al.,1984). Interactions between immune cells are orchestrated by cytokines which are the cellular elements of the immune system responsible for enhancing and coordinating defense against infection. Several key cytokines are involved in the inflammatory response and serve to activate the most appropriate response to the infection. The most beneficial immune responses to different types of infection are shown in Table I.1.

Polymorphonuclear cells are the leukocytes most responsive and capable of controlling bacterial invasion in the normal host (Gadd et al., 1989). Their function is dependent on chemotaxis, margination (adherence), diapedesis, opsonization (el-Falaky et al.,1985), lysozomal enzyme release and production of reactive oxygen intermediates (reviewed in Section I.D.1.d). These functions are activated by products of injured tissue, serum factors, bacterial byproducts (Gadd et al., 1989) and cytokines. Defects in neutrophil function precede infection (Alexander,1986) and correlate with increased risk of sepsis and death (Deitch,1984; Deitch et al.,1986). *In vitro* neutrophil bactericidal activity relates to *in vivo* infection (Dobke et al., 1989; Moran et al., 1988). Infection has been shown to result in inefficient opsonization of foreign pathogens (Zimmerli et al.,1982) which leads to reduced phagocytosis. Corticosteroids released during stress have been shown to impair defenses involved in the oxidative burst of neutrophils such as NADPH oxidase activity (el-Falaky et al., 1985; Rosenthal et al.,1996). Patients with sepsis have been reported to have increased H_2O_2 production (Wakefield et al., 1993) which could result in damage to host tissues.

Natural killer cells, also a component of innate immunity, are important in defense against viruses. They have been shown to lyse infected cells and directly inhibit microbial growth (Levitz et al., 1993; Murphy et al., 1993). The main cytokine responsible for NK activation is IL-12 (Tripp et al.,1993) which is produced by macrophages and PBMCs in response to infection (Bancroft,1993; Chan et al.,1992). IL-12 increases cytolytic activity and promotes IFN-γ synthesis by NK cells which then serves to further activate macrophages.

The gastrointestinal system serves as a primary reservoir for bacteria that can cause life-threatening infections in immunosuppressed patients. Translocation of bacteria from the gut can occur when there is a disruption of normal flora residing there, impaired host defenses, or decreased turnover of the gut mucous and can result in a severe and often deadly sepsis. Sepsis has been associated with anergic T cell responses and reduced proliferation as well as a reduction in T cell numbers. (Burleson et al., 1987; Burleson et al.,1988; O'Mahony et al., 1985).

It has been known for several years that major injury results in a reduction of IL-2 and IFN-γ (Lyons et al., 1997), cytokines produced by the TH1 subset of lymphocytes. A significant increase in IL-4 during infections may inhibit TH1 induction and function. Reduced TH1 responses are detrimental to the ability of the individual to resist septic challenges.

3. Cancer, its Treatment and Immunity

Cancer and therapies used to treat it result in impaired immune responses which are further exacerbated by the patient's nutritional status. Weight loss of 5% is associated with immune deficits and an increased risk of mortality (DeWys et al., 1980). Malnutrition impairs both humoral and cell-mediated immune responses (Gross et al.,1980). For example, malnutrition results in a reduction in the circulating levels of both T-helper ($CD4^+$) and T-suppressor/cytotoxic ($CD8^+$) lymphocytes (Gogos et al.,1990). Supplemental nutrition through enteral feeds has been shown to improve certain immune parameters (Aono et al.,1997; de Oliveira et al.,1997) but these effects may be artifacts of an improved nutritional state rather than a direct effect of enteral nutrition on immune function. During intensive chemotherapy, oral food intake is often decreased because of anorexia, aversion to food, alteration in taste, diarrhea, and malabsorption (Body,1999; Tisdale,1999). TPN was introduced as a source of nutrients for patients undergoing chemotherapy with the goal of improving or maintaining their nutritional status and thereby the clinical outcome. However, no studies to date have demonstrated a beneficial effect of TPN (McGeer et al.,1990) in cancer patients undergoing chemotherapy. A meta-analysis reviewing nutritional intervention using enteral and parental nutrition during chemotherapy concluded that nutritional support in patients receiving chemotherapy is associated with "net harm and no conditions could be clearly defined in which treatment appeared to be of benefit" (McGeer et al., 1990).

Immune surveillance, the ability to detect and destroy tumour cells, is primarily the role of the cellular arm of the immune system, including T-helper and T-suppressor/cytotoxic cells, macrophages, and NK cells (Klein et al.,1993). Tumour cell proliferation, metastatic disease and tumor-host interactions are mediated by complex interactions between the host immune system, growth factors and hormones (Osborne et al.,1987; Pawson,1987). Eicosanoids and cytokines also play significant roles in carcinogenesis (Lundholm et al.,1994; Marnett,1981) and immune regulation of the tumour-mediated response. Nutritional status of the cancer patient has been demonstrated to have a role in the extent of immunosuppression that exists in the cancer patient (Gogos et al.,1998).

It is generally accepted that solid malignancy is associated with depressed immune function (Van Gool et al.,2000). Depressed host immune function during cancer have been reported, including the anti-tumour response by the host to autologous tumour (Black, 1973) and the general immune response to standardized antigens as assayed by *in vitro* and *in vivo* tests (Duckett et al.,1992). Cytotoxic T-cells and NK cells isolated from patients with cancer show reduced tumouricidal toxicity (Brittenden et al.,1996; Klein et al., 1993). NK cells play a key role in mediating non-MHC restricted tumour lysis which is stimulated when tumour cells lose or alter their MHC class I surface molecules and is vital to host defense against malignant cells (Herberman et al.,1981). Macrophages and neutrophils also play critical roles as cytotoxic effector cells against tumours. Activated macrophages release a wide array of biologically active molecules toxic to tumour cells (Denham et al.,1992), such as nitric oxide, which diffuses across cell membranes and inhibits tumour replication (Mills, 1992). Neutrophil responses involve both the production of oxidants and the release of granule constituents (Dallegri et al.,1992) designed to lyse the target cell.

Tumours have developed mechanisms to evade host immune surveillance (Chaux et al.,1997; O'Connell et al.,1999; Van Den Hove et al.,1997). Through release of cytokines and other mediators, tumours can create an environment which suppresses local immune activation such as inducing anergy or programmed cell death (O'Connell et al., 1999; Van Gool et al., 2000). TH1 responses are most effective against tumour cells, however, cytokines and other mediators produced and released by tumours directs tumour-infiltrating cells into a TH2 phenotype, preventing the induction of anti-tumour responses (Heuttner et al.,1995; Huang et al.,1996; Smith et al.,1994).

CD28 is an important co-stimulatory molecule found on T cells that interacts with CD80 on antigen-presenting cells (Allison,1994; June et al.,1994; Linsley et al.,1993). Signalling through the CD28 receptor has been shown to enhance transcription and stabilization of cytokine messages (June et al., 1994). This molecule is also important in overcoming mechanisms used by the tumour to evade host immune responses. For example, expression of CD28 is associated with prevention of T-cell anergy (Allison,1994) and apoptosis of T-cells (Harding et al.,1992).

Current treatment protocols designed to destroy rapidly-proliferating tumour cells also have deleterious effects on host immune functions. In the 1960s, it first became apparent that anti-cancer drugs modulate the immune system, often resulting in transient immunosuppression of both cell-mediated and humoral immunity (Head et al.,1993; Sewell et al.,1993). Reduced numbers of immune cells, with a lower CD4/CD8 ratio (Gogos et al., 1998) is a primary side effect of chemotherapy. Altered phenotypes, reduced NK cell cytotoxicity (Brittenden et al., 1996) and suppressed cell-mediated and humoral immunity have been reported (Head et al., 1993; Sewell et al., 1993). These effects can persist long after chemotherapy has ended.

Surgery is another form of cancer treatment and the postoperative period is a critical time for cancer patients. Monocyte phagocytosis, antigen presentation and superoxide release, B-cell immunoglobulin production, T-lymphocyte proliferation and IL-2 production have been reported to be impaired following surgery (Braga et al.,1996; Grbic et al., 1991; Lennard et al.,1985). The reduction in NK cell cytotoxicity can persist for 2 weeks following surgery (Pollack et al., 1991). Immunosuppression following surgery has been implicated in tumour metastasis formation and development of postoperative septic complications (Heys et al.,1996). Treatment strategies aimed at counteracting these cumulative immunodepressant influences are likely to be of direct benefit to the cancer patient.

Well-designed studies examining the complex relationships among immune function, chemotherapy/radiotherapy and nutrition are currently lacking in the literature. There is a need for supplemental nutrition to optimize nutritional state and restore normal immunological status in an attempt to decrease morbidity, infection rates, and costs (Sriskandan et al.,1997). Further research regarding appropriate nutritional intervention that serves to enhance immunological and metabolic parameters during chemotherapy and other cancer treatments are warranted.

F. Immune Function and Fatty acids

Several studies have demonstrated a relationship between provision of fatty acids and cellular functions (Clandinin et al., 1985; Kinsella,1990) however, few studies have examined immune cell functions as they relate to the composition of individual membrane phospholipids. *In vitro* studies, although limiting in their comparison to humans, have given us insight into how immune function is affected by specific fatty acids. Membrane composition can be rapidly changed in cell culture systems reflecting the fatty acid composition of the media (Anel et al.,1990). Changes in fluidity have been reported to affect transmembrane transport processes (Overath et al.,1970), membrane enzyme activity (Szamel et al.,1981), cell surface antigen expression and cytotoxic potential of T cells (Gill et al.,1980). Well designed animal studies, although rarely done, are useful to examine dietary effects of fatty acids on immune function, particularly those residing in the various lymphoid tissues. Much of the initial clinical evidence for reduced immune function with long chain triglycerides has evolved from studies examining the use of TPN (Alverdy et al.,1992) (Herndon et al.,1987; Kudsk et al.,1992; Moore et al.,1992; Shou et al.,1994)containing Intralipid, 100% fat derived from soybean oil which contains 50 and 9 g/L of linoleic and linolenic acids, respectively (Gottschlich,1992). Further investigations using animals and humans have reported long chain triglycerides to impair both specific (Fischer et al.,1980; Jarstrand et al.,1991) and nonspecific (Nordenstrom et al.,1979) immune responses. Decreased cell mediated immunity (Kinsella,1990), NK cell function (Guillou,1993), macrophage killing (Saba et al.,1979; Sobrado et al.,1985), immunoglobulin synthesis (Salo,1990), complement production (Strunk et al.,1983) and neutrophil function (Fischer et al., 1980; Heine et al.,1999; Jarstrand et al., 1991; Robin et al.,1989) have been reported. These effects may be the reason why high fat regimens increase the incidence of infections (Cleary et al.,1983). Since these observations, specific amounts and types of fats have been shown to have direct or indirect effects on immune function. This review will discuss *in vitro* and animal models as well as human studies that investigate the role of fat in modulating immune functions. Mechanisms proposed for modulation of immune responses by fatty acids will then be discussed.

1. Innate Immune Cells

Macrophage functions include phagocytosis, intracellular killing, presentation of antigen and release of cytokines and prostaglandins. The binding of and affinity for peptides within the major histocompatibility complex has been reported to be affected by PL composition (Roof et al.,1990) and would impact on subsequent cell mediated responses that require recognition of a peptide within this complex for activation. Membrane fluidity plays an important role in phagocytosis (de Pablo et al.,1998) which has been reported to be enhanced after membrane enrichment with unsaturated fatty acids (Calder et al.,1990; de Pablo et al., 1998; Endres,1996). Arachidonic acid is directly involved in phagocytosis and has been implicated in phagosome formation (Lennartz,1999). Both decreases (Eicher et al.,1995) and no effect on phagocytosis (Turek et al.,1994) have been reported with dietary fish oils.

Macrophages readily incorporate EPA and DHA into cellular lipids *in vitro* replacing 25-50% of the 20:4n-6, resulting in 50-65% less PGE_2 being produced (Ogle et al.,1990). Likewise, fish oils from the diet displace 20:4n-6 and 18:2n-6 from macrophage PL (Lokesh et al., 1988) within 4 days in rats (Palombo et al.,1996). Conversely, supplementing the human diet with 5.5 g fish oil per day for 6 weeks has been reported to result in a 7-fold increase in EPA without altering the 20:4n-6 or DHA content in the membranes of neutrophils and monocytes (Lee et al.,1985). However, failure to isolate the individual PL may have masked changes specific to these fractions. Nonetheless, generation of products from 20:4n-6 were reduced in leukocytes with increased EPA content (Lee et al., 1985). Production of oxidative radicals (Fischer et al.,1990; Palombo et al.,1995), release of granules (Virella et al.,1989) and chemotaxis (Payan et al.,1986) of phagocytic cells has been reported to be inhibited with EPA but not 20:4n-6 *in vitro*. However, improved microbial killing by macrophages has been reported in guinea pigs that were fed fish oil compared to 18:2n-6 (Ogle et al., 1993). Conflicting reports regarding effector functions of phagocytic cells could be due to the particular function of the cell of interest that is examined. For example, fish oils have been reported to increase phagocytosis with no effect on intracellular killing capacity (Lokesh et al.,1984; Schroit et al.,1979).

NK cell function are affected by fatty acids (de Pablo et al., 1998; Yaqoob et al.,1994) (Gogos et al., 1990; Guillou,1993) and the metabolites derived from them. NK cells are able to metabolize 20:4n-6 via cyclo- and lipo-oxygenase pathways (Cifone et al.,1993). Increases in the 18:2n-6 content of lymphocytes has been shown to increase the sensitivity of NK cell function to the inhibitory action of PGE_2 (Oxholm et al.,1992). Studies have reported suppressive effects of n-3 fatty acids on NK cell cytoxicity in both rats (Jeffery et al.,1996) and humans (Kelley et al.,1999; Yamashita et al.,1986; Yamashita et al.,1988; Jeffery et al., 1996; Yaqoob et al., 1994) . LTB_4, 5-HPETE and products of the 5-LOX pathway have been shown to enhance NK activity (Chang et al.,1989; Seaman et al., 1984). However, the mechanisms by which NK cells are modulated by products of 20:4n-6 are not fully understood as dietary DHA has been reported to reduce PGE_2 and LTB_4 production but also significantly depress NK activity (Kelley et al., 1999). Alterations in NK activity could be mediated either through membrane compositional changes or by altering the intracellular messages derived from cellular lipids which will be discussed in Section I.F.3.

2. Lymphocytes

Incorporation of n-6 or n-3 fatty acids and changes in the n-6/n-3 fatty acid ratio in immune cells have been shown to affect several functions of lymphocytes.

Polyunsaturated fatty acids suppress mitogenesis to a greater extent than SFA (Calder,1995). High amounts of 18:2n-6 have been reported to inhibit cell mediated immune responses (Johnston,1985; Kinsella,1990). Physiological concentrations of PGE_2 suppress mitogen responsiveness, cytokine production, and cytotoxic T cell generation (Grbic et al., 1991; Kumar et al.,1994). PGE_2 reduces the ability of T cells to produce IL-2 (Peck et al., 1991) and IFN-γ (Snijdewint et al.,1993), cytokines produced by the TH1 subset, depressing cellular immunity and increasing susceptibility to infection (Sammon,1999). These observations support those of Wallace (Wallace et al.,1999) who reported alterations specific to TH1 but not TH2 cells with high intakes of PUFA. Mitogenic responses and IL-2 production of lymphocytes have been reported to be enhanced by the addition of Indomethacin, a cyclooxygenase inhibitor (Calder et al.,1992). Furthermore, abrogation of suppressed proliferation by a large dose of IL-2 to mononuclear cell culture (Grbic et al., 1991) further supports the proposal that decreased lymphocyte proliferation is due to IL-2 inhibition by PGE_2. Alterations in the production of IL-2 would also be expected to impact on NK cell activity and tumoricidal activity of macrophages (Singh et al., 1992).

Reduced lymphocyte proliferation and IL-2 production has also been demonstrated with supplemental n-3 fatty acids in both humans and rats (de Pablo et al., 1998; Endres et al., 1989; Jeffery et al., 1996; Meydani et al., 1991; Soyland et al.,1994; Virella et al.,1991; Yaqoob et al., 1994; Yaqoob et al.,1995), demonstrating that PGE_2 cannot be solely responsible for reduced immune responses. Other studies have reported high doses of dietary fish oil to increase T cell proliferation (Payan et al., 1986). Physiologically relevant levels of n-3 fatty acids fed to rats has been shown to increase the proportion of activated T- and B-cells and macrophages after mitogen stimulation (Robinson et al.,1998). B cell proliferation in response to PWM and their expression of IL-2 receptors are reported to be reduced with EPA *in vitro* (Virella et al., 1989). EPA can directly affect T cells or B cells or can work secondarily to an impairment of accessory cells such as macrophages (Endres et al., 1989). For example, dietary enrichment of EPA has been reported to inhibit the ability of accessory cells to present antigen to T cells, both of the TH1 and TH2 subset (Fujikawa et al.,1992).

Evidence is emerging that fatty acids among the n-3 class can have different effects on membrane composition and immune function. Studies have reported different rates of incorporation of EPA and DHA into cell membranes (Brown et al.,1994). In an attempt to identify the fatty acid responsible for immune modulating effects of fish oil, studies have investigated individual effects of isolated EPA and DHA. Lymphocyte functions reported to be modified by fish oil have not been reported with DHA alone (Kelley et al.,1998). A study comparing dietary linolenic versus DHA and EPA reported differences in immunological effects of n-3 fatty acid derived from plant versus fish oil sources which are likely mediated through changes in membrane composition (Wu et al.,1996).

Conflicting results of studies examining immunological effects of fatty acids may be due to differences in dietary composition between studies. Many animal and human studies have used non-physiological amounts of fatty acids that are often deficient in essential fatty acids. Failure to determine types of acyl groups that the phospholipid are composed of may mask changes in individual PL of fractions comprising cell membranes. In addition, certain cells and tissues may be more susceptible to membrane or functional changes with increasing membrane lipid saturation having varying effects on different cell types. It has been reported that immune cells in different lymphoid compartments respond to lipids in different ways (Jeffery et al.,1999; Yaqoob et al., 1994). For example, rats fed saturated, partially saturated or mixed high fat diets had depressed responses to mitogens in splenocytes but not in the mesenteric lymph nodes (Locniskar et al.,1983). Human work precludes sampling of lymphoid tissues and can only give estimates based on circulating immune cells. Methods used, particularly those from *in vitro* work, often depend on the types of serum being used in the culture systems (Berger et al.,1993; Jeffery et al., 1996; Yaqoob et al., 1994; Yaqoob et al., 1995). Understanding how specific fatty acids, from diet or *in vitro*, can impact on immune function is a complex issue. Many human and animal studies attempting to elucidate this relationship have been poorly designed with major dietary flaws. Carefully controlled feeding trials using physiologically relevant levels of dietary fat and objective outcome measures are necessary to further elucidate lipid effects in health and disease.

3. Mechanisms For Fatty Acid Effects on Immune Function

a) *Fat as an Energy Source*

Lymphocytes utilize glucose and glutamine to a large extent for energy (Ardawi et al.,1984). Intermediates for various biosynthetic pathways are provided by partial oxidation of these substrates. Lactate, glutamate and aspartate are used in purine and pyrimidine synthesis for DNA formation and phospholipid synthesis for membrane biogenesis. Lymphocytes have also been shown to oxidize pyruvate, oleate (Lengle et al.,1978) and long chain PUFA (Yaqoob et al., 1994). It has been demonstrated that lymphocytes residing in lymphoid tissues contain lipoprotein lipase and are able to release and take up of free fatty acids from TG (Calder et al.,1994). PUFA are hydrolyzed to a greater extent than SFA and oleic acid suggesting that lipases of lymphocytes may be substrate specific (Calder et al., 1994).

b) *Intracellular Signals*

Lipids act as intracellular messengers or chemical signaling molecules in immune cells that can activate or attenuate signals through direct or indirect pathways (Graber et al.,1994). Figure I.12 shows the major intracellular pathways generated by lipid components in the membrane. Methylation of PC and PE by transferase enzymes is correlated with Ca^{++} influx, cAMP, PLA_2 activity and effector functions of the cell (Traill et al.,1986). Phosphatidylinositol-bi-phosphate (PIP_2) or PC can be acted upon by phospholipase C, a Ca^{++} dependent protein located at the inner surface of the plasma membrane, to generate inositol triphosphate (IP_3) and diacylglycerol (DAG) that together elicit physiological responses in the cell (Berridge, 1984). PLA_2 acts directly on PI or PC in the membrane to preferentially release 20:4n-6 from the *sn*-2 position (Fonteh et al.,1998; Ramesha et al.,1993). 20:4n-6 activates PLC, guanylate cyclase, adenylate cyclase and PKC. A reduction in PL activity due to replacement of 20:4n-6 by EPA, would result in changes in intracellular messages (Russell et al.,1987), such as synthesis of cytokines, receptors and enzymes. Changes in mRNA expression with dietary fish oil has been demonstrated for several cytokines including IL-2, IL-4, IL-1, IL-6, TNF-α and TGF-β (Fernandes et al.,1994) (Chandrasekar et al.,1994). *In vitro* studies have shown that incorporation of 18:2n-6 in vesicle membranes substantially increases PKC activity (Lester,1990). PGE_2 stimulates adenylate cyclase activity which increases intracellular cAMP concentration and inhibits T cell activation (Grbic et al., 1991).

c) *Membrane Protein Activity*

A plethora of proteins exist in the membrane. Changes in plasma membrane characteristics can change activity of proteins which serve as ion channels, adhesion molecules, receptors or enzymes (de Pablo et al.,2000). Receptor-ligand interactions may be dependent on compositions of specific phospholipids. The insulin receptor is highly dependent on membrane structure. Field et al. (Field et al., 1989) reported a relationship between the amounts and types of fats consumed, phospholipid composition and insulin binding capacity in adipocytes of rats. This study also demonstrated the importance of isolating individual PL to examine their composition, a methodology which is currently lacking in the literature. Adenyl cyclase, which generates cAMP, is very sensitive to changes in membrane fluidity (Kinsella,1990). Cyclic AMP is an important second messenger in the activation or suppression of immune reactions. Its activity increases as phospholipid composition becomes more unsaturated (Brivio-Haugland et al.,1976). Sodium-potassium- adenosine triphosphatase (Na^+-K^+-ATPase) function in lymphocytes is also affected by the degree of unsaturation (Poon et al.,1981). Other enzymes such as Mg-ATPase and γ-glutamyltransferase (Spector et al.,1985) are affected in other types of cells.

Several membrane associated functions of immune cells have been shown to be modulated by membrane changes. Binding of cytokines to their receptors have been reported to be altered (Grimble et al.,1995; Stubbs et al.,1984) with changes in membrane composition. Inhibition of the expression of cell surface molecules such as those involved in adhesion and the MHC complex of human monocytes have been reported with EPA *in vitro* whereas an increase in the expression of human lymphocyte antigen is increased with DHA (Hughes et al.,2000). Expression of the IL-2 receptor has been reported to be inhibited by n-3 fatty acids (Soyland et al., 1994). Many of cell surface proteins are involved in costimulation processes necessary for lymphocyte activation (Hughes et al.,1995). The interactions of cytotoxic T cells with target cell membranes, a necessary interaction to induce effector function, is affected by fluidity of the lipid bilayer of cells (Bialick et al.,1984; Gill et al., 1980) with unsaturated fatty acids increasing and saturated fat decreasing their cytotoxic potential (Tomita-Yamaguchi et al.,1991; Traill et al., 1986).

The extent to which changes in the content of specific fatty acids has the potential to affect subsequent immune responses is not entirely understood. Changes in membrane composition as a result of burn injury or cancer has not been previously investigated. To what extent fatty acids affect membrane dependent proteins or cell surface molecules is not known nor the role of dietary intervention in improving membrane associated functions of immune cells.

d) *Eicosanoids*

Metabolism of 20:4n-6 into leukotrienes, prostaglandins and thromboxanes sustains inflammation required for clearing pathogens (Lennartz,1999). Synthesis of prostaglandins and thromboxanes requires release of 20:4n-6 from membranes and is regulated by several mechanisms. Release of 20:4n-6 from membranes can occur via phospholipase C hydrolysis of PIP_2 resulting in diacylglycerol containing 20:4n-6, and IP_3. Alternatively, 20:4n-6 can be released from phospholipids via phospholipase A_2 activation (Figure I.2). The amount of free 20:4n-6 is regulated by release of 20:4n-6 from membranes (Flower et al.,1976) and acyl-CoA-lysolecithin acyltransferase which incorporates 20:4n-6 into the PL of membranes (Goppelt-Struebe et al.,1986). The final steps in prostaglandin formation requires membrane bound cyclooxygenase and endoperoxidase isomerase as well as cytosolic peroxidase (Herschman,1999).

PGE_2 is generally considered to be an immunosuppressant (Goodwin,1985; Johnston,1985). 20:4n-6 competes with EPA for cyclooxygenase and lipoxygenase pathways (Figure I.1). The biological end products of EPA are several orders of magnitude less potent that their homologous products derived from 20:4n-6 (Kinsella,1990). Supplemental 18:2n-6 from various lipid sources has been shown to increase 20:4n-6 in membranes whereas fish oil reduces 18:2n-6 in membranes suggesting an inhibition of 18:2n-6 desaturation and competition for incorporation into membrane lipids. 20:4n-6 metabolism has been observed to be depressed both in humans and animals receiving fish oil supplementation in the diet (Gerster,1995; Virella et al., 1991; von Schacky et al.,1985). When 20:4n-6 is increased, production of PGE_2 is reported to be increased (Johnson et al.,1997; Kinsella et al.,1990a; Ogle et al., 1990) and vice versa (Lefkowith et al.,1987). Increases in EPA and DHA concentrations in plasma, mononuclear cells and neutrophils have been reported with dietary linolenic acid and EPA (Cerra et al.,1991).

e) *Cytokines*

Macrophages are major producers of PGE_2 and the inflammatory cytokines, IL-1 and TNF-α. PGE_2 has been shown to downregulate the production of both TNF-α and IL-1 (Burch et al., 1989; Goodwin,1985; Kawakami et al., 1986; Kinsella et al., 1990b; Kunkel et al., 1986). There is an inverse relationship between 20:4n-6 content of lymphocytes and their production of IL-1 and TNF-α (Hubbard et al.,1993) which could be a result of increased PGE_2 synthesis. N-3 fatty acids are considered to be anti-inflammatory in a variety of conditions (Zurier,1993). The production of pro-inflammatory cytokines such as IL-1 and TNF-α have been shown to be modulated by n-3 fatty acids (de Pablo et al., 1998; Endres,1996), although studies have been conflicting (Blok et al.,1992; Blok et al.,1996; de Pablo et al., 1998; Endres,1996; Hardardottir et al.,1991). Diets containing n-3 fatty acids from both plant and animal sources (Caughey et al.,1996) have been shown to reduce the production of inflammatory cytokines (Caughey et al., 1996; Endres et al., 1989; Kremer et al.,1990; Meydani et al., 1991). Reduced 20:4n-6, PGE_2, IL-1 and TNF-α production by monocytes from healthy humans fed high fish oil diets has been reported (Endres et al., 1989) whereas supplementing the diet of healthy males with 20:4n-6 (1.5 g/day) has been shown to result in significant increases in the *in vitro* secretion of both PGE_2 and LTB_4 while not changing TNF-α, IL1, IL-2, IL-6 expression. (Kelley et al., 1999). These studies demonstrate the complexities of the relationships between dietary lipids and cytokine production and suggest other mechanisms apart from 20:4n-6 metabolites in mediating cytokine changes in response to dietary fatty acids. Differences between studies may be due to different diet compositions, length of time on the study diet or the particular immune cell examined. In addition, effects observed during states of stress may be different than those observed in healthy humans.

4. Neutrophils

The major neutrophil functions, adherence, chemotaxis, degranulation and decreased lysozomal content are membrane associated phenomenon (Deitch,1984) and therefore neutrophils serve as a good example to illustrate and review how changes in fatty acid composition of the membrane can affect cellular function. The receptors on the surface of the neutrophils are part of the membrane lipid environment. A major receptor found on the surface of neutrophils, the FC-γ-III receptor (Hundt et al.,1992) is linked via glycosyl-phosphatidylinositol in neutrophils. It is important in antibody dependent cell cytotoxicity, ligand binding, phagocytosis, immune complex clearance and respiratory burst activity. Adherence to the endothelium requires membrane receptors located within the membrane lipid environment. Membrane fluidity is an important regulator of phagocytosis (de Pablo et al., 2000). Phagocytosis requires rapid synthesis of new membrane that would consume significant amounts of essential fatty acids to enlarge and engulf the microorganism. The NADPH oxidase system is known to be associated with PLC, and PKC, key intracellular regulators of lipid metabolism activated by the cleavage of phosphatidylinositol. Arachidonic acid is an intracellular activator of the NADPH oxidase (Sakata et al.,1987). Furthermore, products of 20:4n-6 metabolism are important modulators of leukocyte functions. For example, PGE_1, E_2 and prostacyclin reduce chemotaxis, phagocytosis, motility, margination and adherence of neutrophils to surfaces whereas the products of the lipoxygenase pathway are potent chemoattractants for neutrophils (Boxer et al.,1980; Penneys et al.,1977; Samuelsson,1983; Spagnuolo et al.,1980). LTB_4 stimulates adherence to endothelium, chemokinesis, degranulation and aggregation of neutrophils (Bray et al.,1983). Similarily, 18:2n-6 enrichment of cell membranes has been reported to increase the oxidative burst of neutrophils (Gyllenhammar et al.,1990; Sassen et al.,1993). Neutrophils produce and respond to cytokines that have been reported to be altered by dietary fat (Cassatella et al.,2000). TNF-α causes degranulation, activation of the respiratory burst and increased microbiocidal actvity of neutrophils (Beutler,1988; Dinarello et al., 1989; Sherry et al., 1988). Therefore, intracellular signals, enzymes, receptors and effector function can be affected by alterations in lipid composition of neutrophil membranes.

5. Clinical Trials Investigating Fatty Acids and Immune Function in Disease

Malnutrition increases risk of infection through its effects on immune function (Chandra et al.,1994; Hulsewe et al.,1999; Keusch,1998; Woodward,1998). Infections promote malnutrition, creating a complex cycle of immunosuppression and malnutrition (Scrimshaw et al.,1997) that if not addressed, can lead to subsequent complications in recovery. Although immunological measures were introduced as part of assessing nutritional status in the 1970s (Bistrian et al.,1975), it was not until 1980 that the first prospective randomized clinical trial was reported that clearly demonstrated that nutritional intervention could improve patient survival and reduce infectious incidences (Alexander,1980). Early nutritional support consisting of sufficient calories and protein have proven themselves to be of benefit and are now a common feeding protocol in critically ill patients (Shirani et al.,1996). The past 25 years have resulted in an explosion of scientific literature defining the role of specific nutrients on immune function during different physiological states. Recent advances in the understanding of the role of specific nutrients in modulating immunity and inflammation have stimulated the development of nutritional regimens containing supplemental nutrients designed to improve recovery from trauma and critical illness. The specific nutrients or combination of nutrients that are most immunologically and metabolically beneficial during critical illness are not yet established. The focus of this review will be fatty acids, and those where immune or outcome measures were examined in various states of stress. In many cases, however, other "immuno-enhancing" nutrients have also been added to these formulations.

Extrapolating information regarding beneficial effects on immune function, infections and clinical outcomes based on the clinical trials performed to date is difficult (Table I.2). Randomized clinical trials with prospective definitions of methods and outcome measures that are performed in a double blind fashion provide the best evidence, however, the few of these that exist should be of higher quality (Solomon et al.,1998). For example, most of the diet comparisons were not isonitrogenous between experimental groups. Lack of well-defined objective outcome measures, such as the definition of infection are lacking and vary between studies. Rarely are specific immune functions related to clinical outcomes. Immune cell numbers is not necessarily a useful measure unless function is also assessed. Multicentre trials are useful in that various treatment protocols are subject to the same diet treatments therefore, one can presume that the observed effect is mediated through diet when criteria for discharge, subjectivity of infection, antibiotic usage and methodologies may differ between institutions.

Furthermore, the large number of patients enrolled in these trials allow for the measurement of mortality rates.

Of the studies discussed in Table 1.2, a significant reduction in length of hospital stay was reported in 4/12 studies but only 2 of those used isonitrogenous formulations. Four studies reported a reduction in infections with the supplemented diet and 2 did not. Pneumonia and wound infection were reduced in 3/12 studies versus 5/12 that showed no effect on these parameters. Of the immune parameters examined, cytokines do not appear to be remarkably affected by the supplements used. For intervention trials with burn patients, low fat seems to be of benefit in reducing infectious incidence, and length of hospital stay, however, it does not appear to alter immune parameters measured in these studies. In patients undergoing surgery for cancer, results are more varied. When the supplemented diet contained more nitrogen, there does appear to be benefits, however, McCarter *et al.* (1998) found no benefits but rather outcomes may have been worsened although not statistically different. Studies with more patients might help clarify these findings. High doses of fish oil appear to be beneficial in reducing immune responses associated with cachexia and likely mediate these effects via mechanisms discussed in Sections I.C.c and I.E.b and I.F.

Expensive formulations are not warranted unless it can be conclusively demonstrated that length of hospital stay is reduced, which generally translates into fewer infections and complications throughout recovery. A study that compared a hospital made diet to an expensive commercial diet demonstrated no differences in outcome measures in a small number of patients (Dhanraj et al.,1997). On the other hand, a large, multicentre trial, reported a supplemented commercial diet to reduce treatment costs due to a lower frequency of postoperative infectious and wound complications (Senkal et al.,1997).

Feeding formula containing multiple "immunonutrients" has been reported to be beneficial, however, attempts to determine the specific nutrient responsible for a particular outcome is not easily deduced. Due to the inclusion of different immune enhancing nutrients in the diets, formulations of the diets, and lack of similar nitrogen contents, it is difficult to isolate the specific nutrient or combinations responsible for improvement. Furthermore, one nutrient may work synergistically with others to produce an effect over and above what would be observed with a single nutrient alone. In addition to the benefits of the specific nutrients described in the studies, the importance of vitamins and trace minerals cannot be overlooked. Burn, surgery and cancer patients have increased metabolic and immune requirements which require a significant amount of cofactors for optimal biochemical processes. In addition, additional micronutrients are required in wound healing processes (Berger et al.,1998; Meyer et al., 1994).

When the stress response is activated, and several different metabolic and inflammatory mediators are released, it is unlikely that nutritional intervention can reverse this process. However, nutrition is necessary to maintain gut integrity and maintain nutritional status. It is possible that supplemental nutrients such as fatty acids are required at higher doses during acute/severe disease states. Specialized formulas containing defined nutrients are warranted in prolonged disease states such as recovery from burn injury, chemotherapy and cachexia.

G. Summary

Profound alterations in fatty acid metabolism have been demonstrated in disease states such as burn injury and cancer. These alterations may change the way essential fatty acids are metabolized and distributed to various tissues in the body including the immune system. The essential fatty acids available to the tissues during chemotherapy, cancer and recovery from burn injury has not been investigated. Fatty acids have been shown to have significant effects on immune functions in a variety of cell types. Many of these effects are modulated by changes in fatty acid composition of cells. The phospholipid fatty acid composition of immune cells has not been examined in patients with cancer or major burns. In spite of several studies investigating the role of fatty acids on immune function, to date, objective measures by which to measure efficacy of dietary intervention with specific fatty acids have not been defined. Determining the extent to which dietary fatty acids get incorporated into tissues such as those of the immune system might provide an objective and consistent measure of efficacy of intervention with specific fatty acids. If changes could be related to immune function, infectious incidence and clinical outcomes, then membrane composition would provide a useful clinical biomarker. Furthermore, an understanding of how fatty acid metabolism alters membrane composition in diseases characterized by immunosuppression will be useful in the design of clinical diets aimed at up or down regulating immune responses in these disease states.

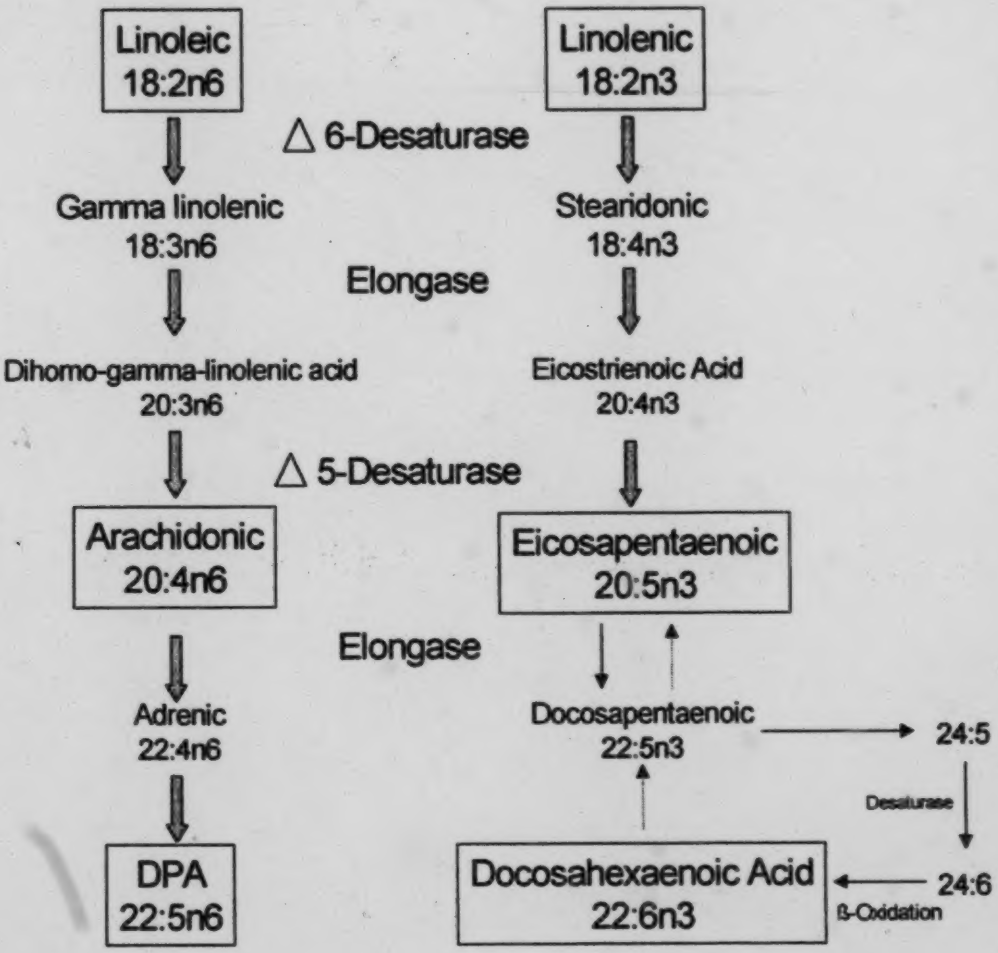

Linoleic
18:2n6
Linolenic
18:2n3
△ 6-Desaturase
Gamma linolenic
18:3n6
Stearidonic
18:4n3
Elongase
Dihomo-gamma-linolenic acid
20:3n6
Eicostrienoic Acid
20:4n3
△ 5-Desaturase
Arachidonic
20:4n6
Eicosapentaenoic
20:5n3
Elongase
Adrenic
22:4n6
Docosapentaenoic
22:5n3
24:5
Desaturase
DPA
22:5n6
Docosahexaenoic Acid
22:6n3
24:6
ß-Oxidation

FIGURE I.1: Conversion of dietary essential linoleic and linolenic acid into other n-6 and n-3 fatty acids by elongase and desaturase enzymatic pathways.

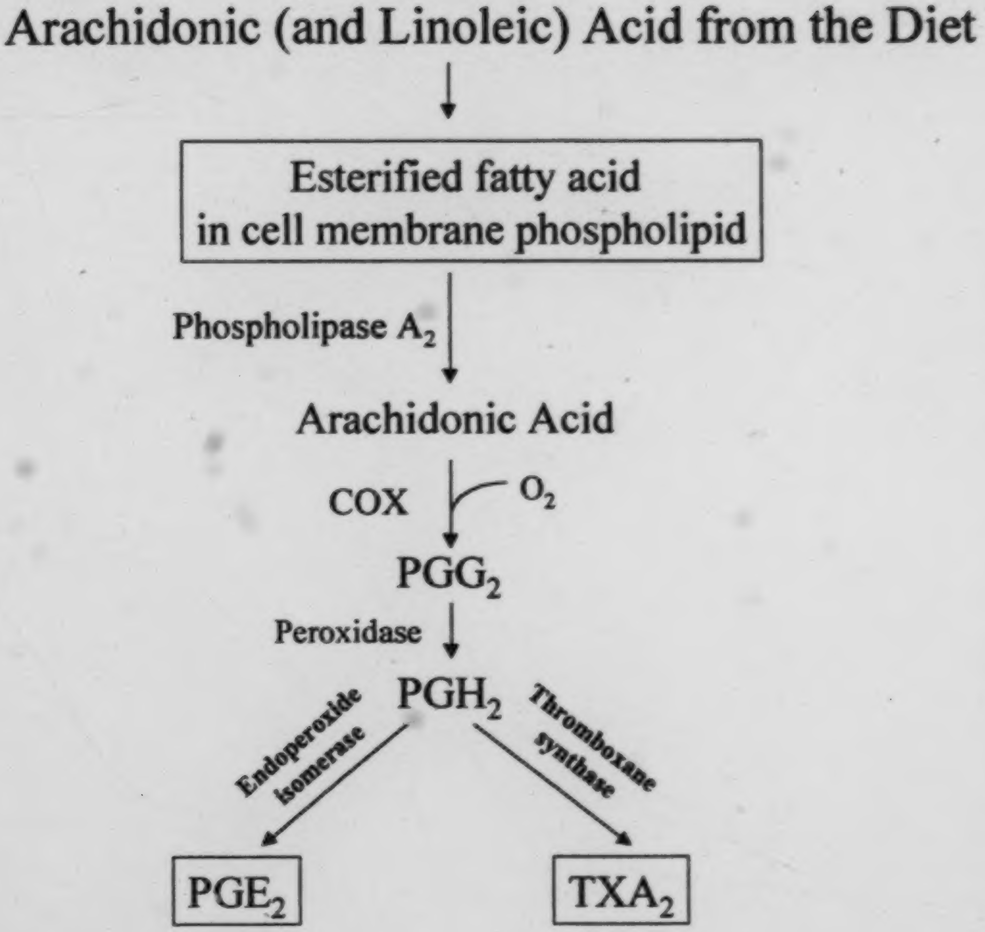

Arachidonic (and Linoleic) Acid from the Diet
Esterified fatty acid in cell membrane phospholipid
Phospholipase A2
Arachidonic Acid
COX
O2
PGG2
Peroxidase
PGH2
Endoperoxide isomerase
Thromboxane synthase
PGE2
TXA2

FIGURE I.2: Synthesis of 2-series prostaglandins and thromboxane A_2 from arachidonic acid by reactions catalyzed by cyclooxygenase and peroxidase enzymes. Arachidonic acid is released from the membrane by PLA_2 and is subsequently converted to PGE_2 and TXA_2 by endoperoxide (E) isomerase and thromboxane synthase, respectively.

Abbreviations: COX: Cyclooxygenase, PG: Prostaglandin; TX: Thromboxane

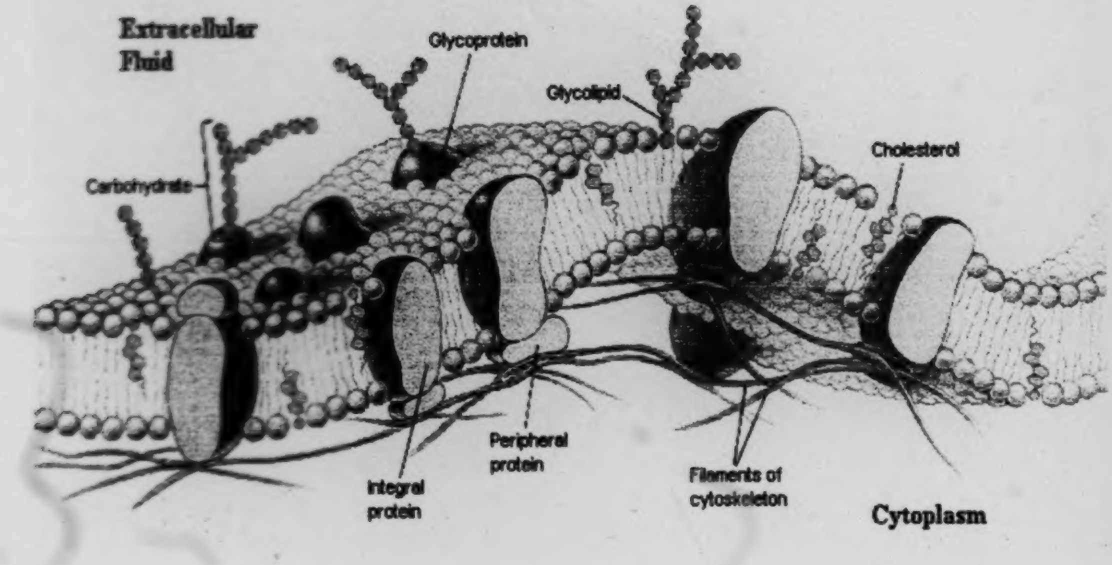

Extracellular Fluid
Glycoprotein
Glycolipid
Cholesterol
Carbohydrate
Peripheral protein
Integral protein
Filaments of cytoskeleton
Cytoplasm

FIGURE I.3: A Biological membrane composed of a bilayer of PL with integral and peripheral proteins throughout. Carbohydrate moeities can be attached to proteins or lipids to form glycoproteins and glycolipids, respectively. Cholesterol is also an important membrane component and governs fluidity. Proteins may have specific requirements for fatty acids or PL in the membrane environment *(Source: http://www.people.virginia.edu/~rjh9u/cellmemb.html reprinted with permission as modified by Robert J. Huskey, University of Virginia).*

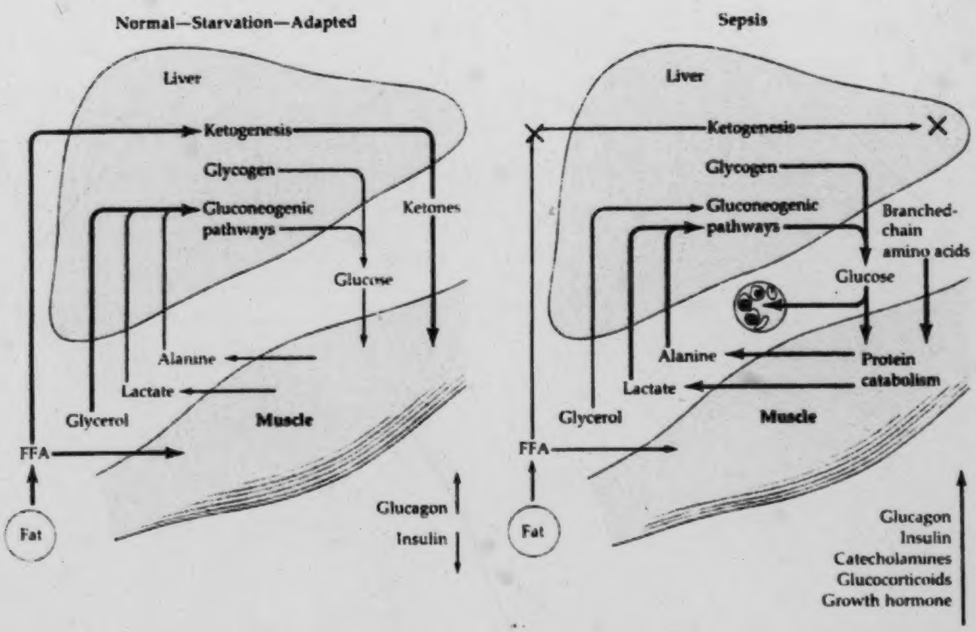

Normal—Starvation—Adapted
Liver
Ketogenesis
Glycogen
Gluconeogenic pathways
Ketones
Glucose
Alanine
Lactate
Glycerol
Muscle
FFA
Fat
Glucagon
Insulin
Sepsis
Liver
Ketogenesis
Glycogen
Gluconeogenic pathways
Branched-chain amino acids
Glucose
Alanine
Protein catabolism
Lactate
Glycerol
Muscle
FFA
Fat
Glucagon
Insulin
Catecholamines
Glucocorticoids
Growth hormone

FIGURE I.4: A summary of the major metabolic changes that occur during the stress response compared to the normal starvation response. During simple starvation, fat mobilization and ketogenesis provide energy, thereby sparing body protein and decreasing gluconeogenesis. In contrast, sepsis causes body proteins to be catabolized at an accelerated rate, gluconeogenesis is stimulated, while ketogenesis is inhibited and fat mobilization is decreased. Increased responses during starvation and sepsis are shown by the heavy arrows. (*Reprinted with permission. Source: Groff, 1995*).

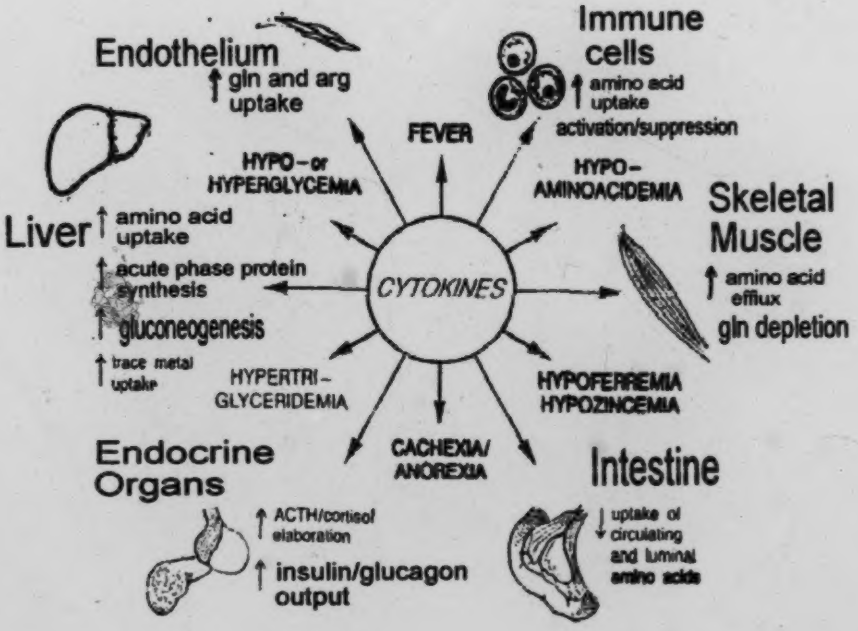
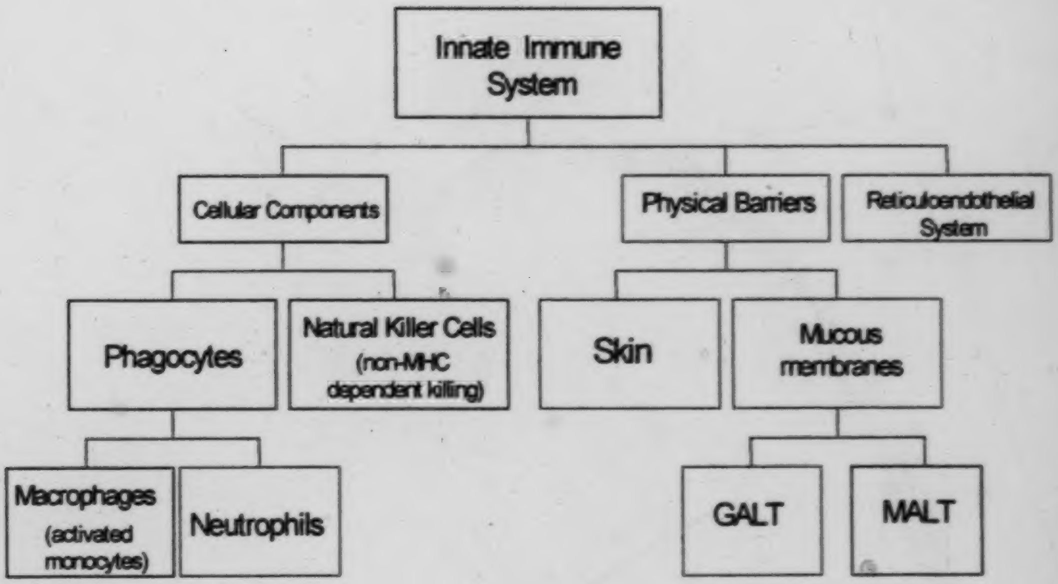
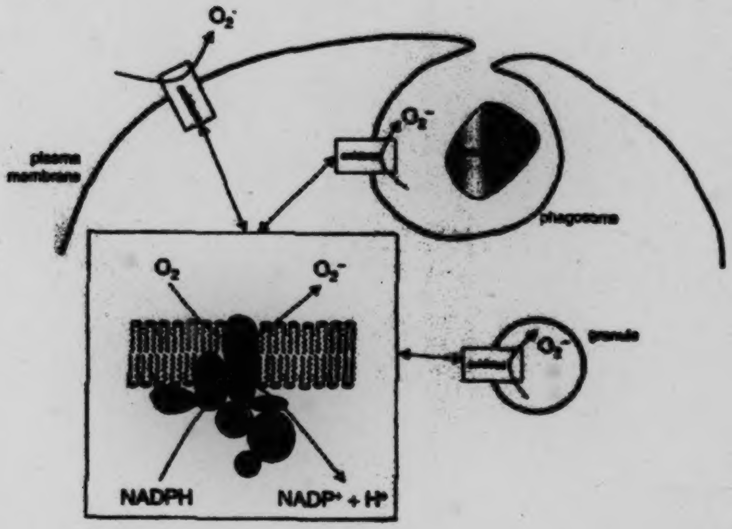

Endothelium
↑ gln and arg uptake
Immune cells
↑ amino acid uptake
activation/suppression
FEVER
HYPO- or HYPERGLYCEMIA
HYPO- AMINOACIDEMIA
Liver ↑ amino acid uptake
↑ acute phase protein synthesis
↑ gluconeogenesis
↑ trace metal uptake
CYTOKINES
Skeletal Muscle
↑ amino acid efflux
gln depletion
HYPERTRI- GLYCERIDEMIA
HYPOFERREMIA
HYPOZINCEMIA
CACHEXIA/ ANOREXIA
Endocrine Organs
↑ ACTH/cortisol elaboration
↑ insulin/glucagon output
Intestine
↓ uptake of circulating and luminal amino acids

FIGURE I.5: The effect of pro-inflammatory cytokines on various tissues in the body (*Reprinted with permission. Source: Souba et al., 1994*).



Innate Immune System
Cellular Components
Physical Barriers
Reticuloendothelial System
Phagocytes
Natural Killer Cells (non-MHC dependent killing)
Skin
Mucous membranes
Macrophages (activated monocytes)
Neutrophils
GALT
MALT

FIGURE I.6: Components of the Innate Immune System (Pratt et al.,2000)


O_2^-
plasma membrane
O_2^-
phagosome
O_2
O_2^-
O_2^-
granule
NADPH
NADP⁺ + H⁺

$$\begin{tikzcd}[column sep=small]
\mathrm{NADPH} + 2O_2 + H^+ \arrow[rr] & & \mathrm{NADP}^+ + 2O^{2-} + 2H^+ \arrow[dl] \arrow[dr] & \\
& H_2O_2 + H_2O & & OH^-
\end{tikzcd}$$

FIGURE I.7: Phagocytosis activation of the NADPH oxidase multi-component enzyme complex and the reaction it catalyzes *(Reprinted with permission as adapted from Dahlgren, 1999.)*.

DOT PLOT
Neutrophils gated

Mean Channel
Fluorescence
(FL-1)

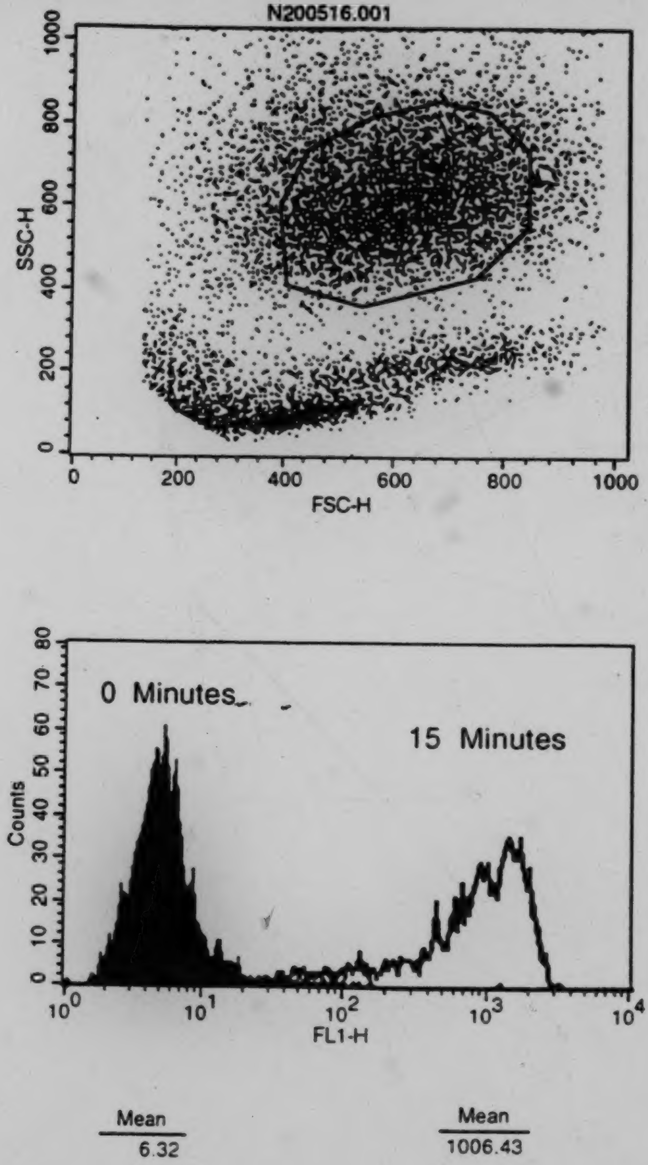


FIGURE I.8: Analysis of the respiratory burst using flow cytometry. Whole blood deplete of red blood cells are incubated with dihydrorhodamine and the mean channel fluorescence (MCF; FL-1) of unstimulated cells and cells stimulated with PMA are analyzed using flow cytometry. Neutrophils are gated according to their side and forward scatter characteristics. The change in oxidative burst is measured by the stimulated MCF/ unstimulated (0 minute) MCF.

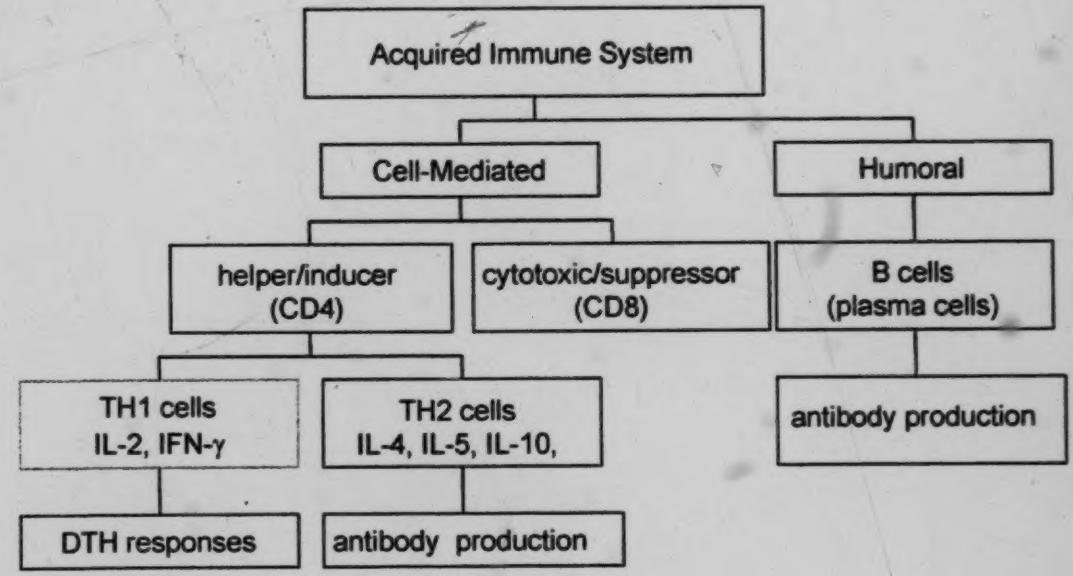

Acquired Immune System
Cell-Mediated
Humoral
helper/inducer (CD4)
cytotoxic/suppressor (CD8)
B cells (plasma cells)
TH1 cells IL-2, IFN-γ
TH2 cells IL-4, IL-5, IL-10,
antibody production
DTH responses
antibody production

FIGURE I.9: Components of the Acquired Immune System (Pratt et al., 2000)

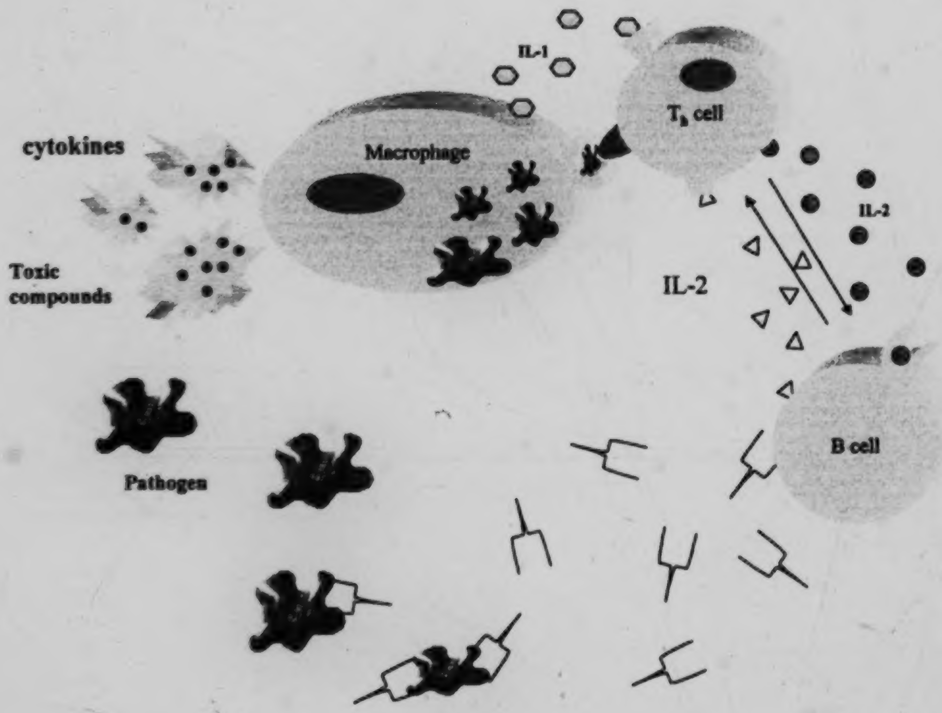

IL-1
T_h cell
cytokines
Macrophage
IL-2
Toxic compounds
IL-2
B cell
Pathogen

FIGURE I.10: The interactions between innate and acquired immune cells in the elimination of pathogens *(Adapted from Pratt, 2000)*. A macrophage presents an antigenic peptide within the MHC II complex to T-helper cells which then produce cytokines to recruit and direct B cells to produce antibodies effective against an infecting agent. The macrophage can also produce cytokines or oxidative molecules that are directly toxic to an infecting agent.

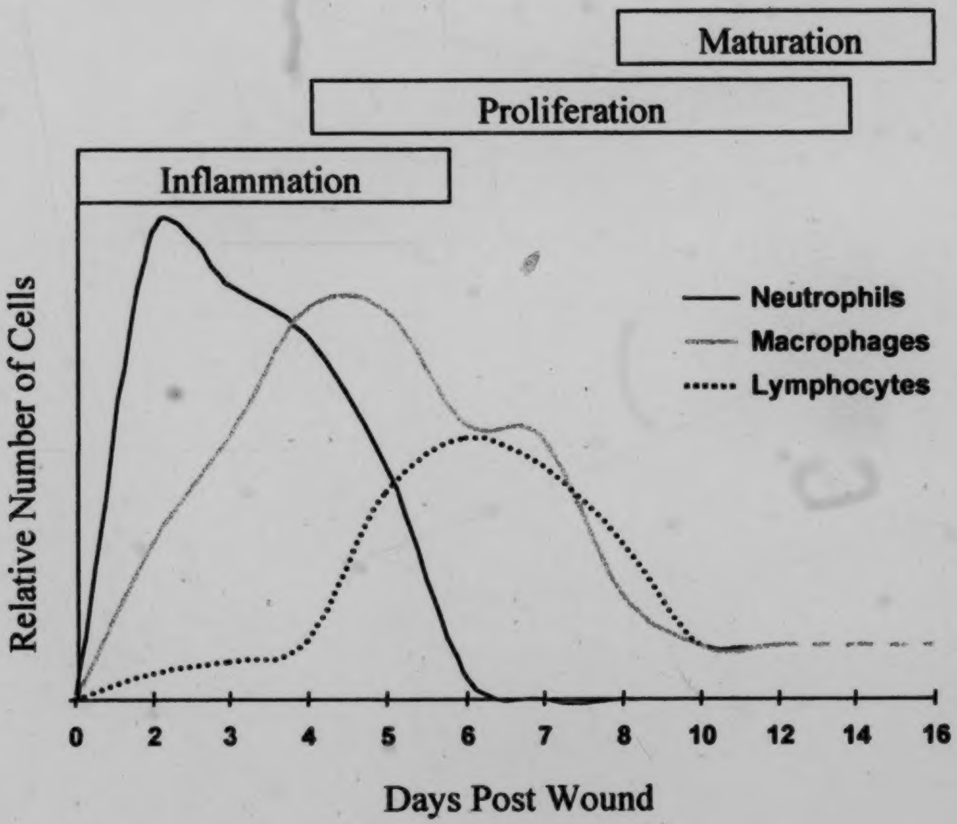


FIGURE I.11: The role of immune cells in the progression of wound healing. Neutrophils are the first cells to appear within 2 hours of injury. Macrophages are next to infiltrate the wound area and produce cytokines and other factors that promote wound healing. Macrophages and lymphocytes remain in the wound until it is healed. *(Adapted with permission from DiPietro, 1995).*

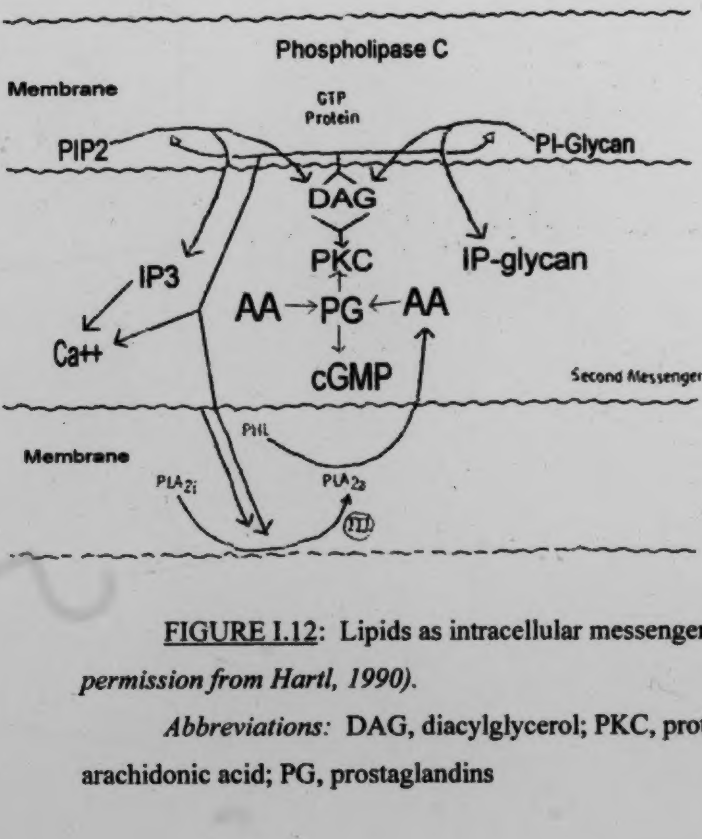

Phospholipase C
Membrane
GTP Protein
PIP2
PI-Glycan
DAG
PKC
IP-glycan
IP3
AA
PG
AA
Ca++
cGMP
Second Messenger
PHL
Membrane
PLA2i
PLA2a

FIGURE I.12: Lipids as intracellular messengers. *(Adapted with permission from Hartl, 1990).*

Abbreviations: DAG, diacylglycerol; PKC, protein kinase C; AA, arachidonic acid; PG, prostaglandins

INFECTION TYPE	EXAMPLES	IMMUNE RESPONSE	MOLECULES & CELLS INVOLVED
Viruses (Ploegh,1998)	HIV Influenza Rhinovirus	1. Humoral to block initial infection and for recruitment of other cells. 2. Cell mediated response is most effective after cells are infected	Antibodies: agglutination, opsonization IFN-γ: anti-viral cytokine NK and cytotoxic T cells: directly cytoxic to virally infected cells
Parasites (Sher, 1992)	Trichinella Helminths	Low levels as there is no replication inside host cells	B cells located within GALT
Protozoa (Mahmoud, 1989)	malaria	1. Bloodborne cleared by humoral antibodies 2. Infected cells destroyed by cell mediated functions	Antibodies: agglutination, opsonization T cells: cytotoxic killing
Intracellular Bacteria (Kaufmann, 1993)	*Listeria monocytogenes* *Mycobacterium tuberculosis*	1. Cell mediated (DTH) response 2. TH1 type of immune response and cytokine pattern	IFN-γ activation of macrophages Macrophages: phagocytosis Neutrophils: ingestion and enzymatic killing
Extracellular bacteria	*Streptococcus pnemoniae*	Humoral components located in lymph nodes of the gastrointestinal and respiratory tracts	Antibodies: opsonization, complement activation and recruitment of other immune cells Phagocytes: killing
Sepsis (Systemic inflammatory response system) (Pajkrt, 1996)	Endotoxin, LPS	Dysregulated inflammatory response	Neutrophils: release oxidative compounds Macrophages: cytokine production

TABLE I.1: Infections and the types of immune responses most effective in eliminating them (Pratt, 2000).

TABLE I.2: Clinical trials investigating supplementation of fatty acids in burns and cancer

Reference	Fat studied	Other nutrients	Compared to	Patient group	Design	Benefits/Changes	No effects
Gottschlich *et al.* (1990)	Shriner's tube feed recipe (11% total fat 50% n-3, 50% safflower)	Arginine Vitamin A Vitamin C Zinc	Osmolite and Traumacal (lower fat in exp diet) Isonitrogenous	Burns (n=50)	Double blind, prospective, randomized,	↓ wound infection ↓ LHS ↓ pnemonia ↓ infectious complications ↓ loss of LBM ↓ Mortality ($p<0.06$)	Sepsis Weight loss Days requiring antibiotic Nutritional index and acute phase proteins Functional immune tests
Saffle *et al.* (1997)	Impact n-3 fatty acids (1.3g EPA and 0.45g DHA)	Arginine RNA	Replete (high protein, + gln and 30% fat) Not isonitrogenous	Burns (n=50)	Randomized	Replete had ↓ infectious and noninfectious complications (not significant)	LHS Mortality Infectious complications Days of ventilator support
Garrel *et al.* (1995)	Low fat with and without n-3 fatty acids	N/A	Without n-3 fatty acids and higher fat formulas	Burns (n=43)	Prospective, randomized	↓ pneumonia ↑ respiratory status ↑ nutritional status ↓ LHS	Cytokines N-balance
Daly *et al.* (1992)	Impact n-3 fatty acids (2.2 g n-3/day; (1.7g EPA/DHA)	Arginine RNA	Osmolite Not Isonitrogenous	Gastric Cancer Surgery (n=85)	Randomized	↑ lymphoycte blastogeneisis ↓ infectious and healing complications ↓ LHS	Mortality Pnemonia Infection Opsonic index serum C3 Other complications
Kemen *et al.* (1995)	Impact n-3 fatty acids	Arginine, RNA	Placebo Not isonitrogenous	Gastric Cancer Surgery (n=42)	Prospective, randomized, placebo, double blind	↑ HLA-DR expression ↑ CD3, CD4 and B cells, ↑ IFN-γ concentration	Albumin and transferrin IgM
McCarter *et al.* (1998)	n-3 fatty acids (2.6 g/d EPA and DHA)	Arginine Vitamin fortified	standard formula +/- arginine Isonitrogenous	Gastric Cancer Surgery (n=48)	Randomized, double blind	Nonsupplemented fared better on all measures (not statistically significant)	Lymphoycte mitogenesis Cytokines Infections Infectious complications LHS

Braga *et al.* (1996)	Impact n-3 fatty acids	Arginine RNA	standard enteral formula and TPN Isonitrogenous	Gastric Cancer surgery (n=62)	Randomized, prospective	↓ severe infectious incidence ↑ Phagocytosis, DTH ↑ IL-2 receptor ↓ IL-6 ↓ LHS	CD4, CD8, CD4/CD8
Bower *et al.* (1995)	Impact n-3 fatty acids (2.6 g EPA/DHA)	Arginine RNA Vitamins E, β-carotene Selenium	Osmolite Not isonitrogenous	ICU patients (n=296)	Multicenter, prospective, randomized, double blind	↓ LHS ↓ LA and ↑ DHA, EPA ↑ plasma amino acids	Immune function Septic complications N-balance
Cerra *et al.* (1991)	Impact n-3 fatty acids	Arginine RNA	Osmolite	Surgical ICU hyper-metabolic	Randomized, blind, prospective	↑ blastogenesis	Clinical outcome N-balance visceral protein markers
Moore *et al.* (1994)	"Immune-Aid" 1.1 g/L n-3 fatty acids	Glutamine Arginine	Vivonex Not isonitrogenous	Trauma patients (n=114)	Multicenter, Controlled, prospective,	↑ plasma amino acids, ↓ abdominal abcesses ↓ MOF ↑ CD3 and CD4 cells ↓ LHS (not significant)	Serum albumin, transferrin, visceral and acute phase proteins Blastogeneisis Cytokines
Gogos *et al.* (1998)	EPA and DHA (18g/day)	Vitamin E	40 days	Cancer (n=60)	Randomized, prospective	↑ CD4/CD8 ratio ↑ TNF production in malnourished subgroup ↑ Karnofsky status	IL-1 or IL-6 production Body weight Serum albumin, transferrin
Purasiri *et al.* (1994)	γ-linolenic acid, EPA and DHA (4.8 g/day)	N/A	6 months	Local/ advanced colorectal cancer (n=30)	N/A	↓ proliferation ↓ CD4 and CD8 ↓ NK number and activity ↓ TNF, IL-6, IFN, IL-1, IL-4 in serum	
Weimann *et al.* (1998)	n-3 fatty acids Impact	1.25 g/100 ml arginine, RNA	N/A	Severe trauma patients (n=29)	Prospective, randomized, double blind (n=32)	↓ MOF, SIRS ↓ C-reactive protein, fibrinogen	CD4/CD8 IL-2 receptor Infection Mortality LHS

TABLE I.2 cont'd: Clinical trials investigating supplementation of fatty acids in burns and cancer

Table I.2 cont'd: **Clinical trials investigating the effects of supplementing fatty acids and other nutrients in clinical diets for thermally injured and cancer patients. The amount and type of fats used in each intervention, the design of the study and the patient group are indicated. Immune measures, if performed, are indicated in the column labeled benefits/changes.**

G. References

Aarsland A, Chinkes D, Wolfe RR et al. Beta-blockade lowers peripheral lipolysis in burn patients receiving growth hormone. Rate of hepatic very low density lipoprotein triglyceride secretion remains unchanged. Ann Surg 223: 777-787, 1996.

Abbas, AK, Lichtman, AH, and Pober, JSB cell activation and Antibody Production In: Cellular and Molecular Immunology, 2, Abbas, A K pp. 187-204.W.B. Saunders, Pennsylvania ,1994.

Alam R. A brief review of the immune system. Prim Care Clin Off Pract 25: 727-738, 1998.

Alexander JW. Immunological responses in the burned patient. J Trauma 19: 887-889, 1979.

Alexander JW. The role of host defense mechanisms in surgical infections. Surg Clin North Am 60: 107-116, 1980.

Alexander JW. William A. Altemeier lecture. Nutrition and infection. New perspectives for an old problem. Arch Surg 121: 966-972, 1986.

Alexander JW, Ogle CK, Stinnett JD et al. A sequential, prospective analysis of immunologic abnormalities and infection following severe thermal injury. Ann Surg 188: 809-816, 1978.

Allison JP. CD28/B7 interactions in T cell activation. Curr Opin Immunol 6: 414-419, 1994.

Alverdy JC and Burke D. Total parenteral nutrition: iatrogenic immunosuppression. Nutrition 8: 359-365, 1992.

Anel A, Naval J, Gonzalez B et al. Fatty acid metabolism in human lymphocytes. II. Activation of fatty acid desaturase-elongase systems during blastic transformation. Biochim Biophys Acta 1044: 332-339, 1990.

Angel J, Colard O, Chevy F et al. Interleukin-1-mediated phospholipid breakdown and arachidonic acid release in human synovial cells. Arthritis Rheum 36: 158-167, 1993.

Antonacci AC, Reaves LE, Calvano SE et al. Flow cytometric analysis of lymphocyte subpopulations after thermal injury in human beings. Surg Gynecol Obstet 159: 1-8, 1984.

Aono K, Isobe K, Kiuchi K et al. In vitro and in vivo expression of inducible nitric oxide synthase during experimental endotoxemia: involvement of other cytokines. J Cell Biochem 65: 349-358, 1997.

Ardawi MS and Newsholme EA. Metabolism of ketone bodies, oleate and glucose in lymphocytes of the rat. Biochem J 221: 255-260, 1984.

Arturson G, Hamberg M, Jonsson CE. Prostaglandins in human burn blister fluid. Acta Phys Scand 87: 270-276, 1973.

Azzara A, Carulli G, Sbrana S et al. Effects of lysine-arginine association on immune functions in patients with recurrent infections. Drugs Exp Clin Res 21: 71-78, 1995.

Baker CC, Yamada AH, Faist E et al. Interleukin-1 and T cell function following injury. J Burn Care Rehab 8: 503-508, 1987.

Bancroft GJ. The role of natural killer cells in innate resistance to infection. Curr Opin Immunol 5: 503-510, 1993.

Barber MD, Ross JA, Preston T et al. Fish-oil enriched nutritional supplement attenuates progression of the acute-phase response in weight-losing patients with advanced pancreatic cancer. J Nutr 129: 1120-1125, 1999.

Barbul A, Breslin RJ, Woodyard JP et al. Arginine enhances wound healing and immune responses in humans. Surg 108: 331-337, 1990.

Barbul A, Breslin RJ, Woodyard JP et al. The effect of in vivo T helper and T suppressor lymphocyte depletion on wound healing. Ann Surg 209: 479-483, 1989.

Barbul A and Regan MC. Immune involvement in wound healing. Otolaryngol Clin North Am 28: 955-968, 1995.

Barton RG. Nutrition support in critical illness. Nutr Clin Pract 9: 127-139, 1994.

Beck SA, Smith KL, Ross JA et al. Anticachectic and antitumour effect of eicosapentaenoic acid and its effect on protein turnover. Cancer Res 51: 6089-6093, 1991.

Beck SA and Tisdale MJ. Production of lipolytic and proteolytic factors by a murine tumor producing cachexia in the host. Cancer Res [illegible]: 5919, 1987.

Bengtsson A. Cascade system activation in shock. Acta Anaesth Scand Supplementum. 98: 7-10, 1993.

Benhaim P and Hunt TK. Natural resistance to infection: leukocyte functions. J Burn Care Rehab 13: 287-292, 1992.

Berger A, German JB, Chiang BL et al. Influence of feeding unsaturated fatty acid on growth and immune status of mice. J.Nutr. 123:225-233, 1993

Berger MM, Spertini F, Shenkin A et al. Trace element supplementation modulates pulmonary infection rates after major burns: a double-blind, placebo-controlled trial. Am J Clin Nutr 68: 365-371, 1998.

Berlin E, Bhathena SJ, Judd JT. Dietary fat and hormonal effects on erythrocyte membrane fluidity and lipid composition in adult women. Metabol 38: 790-796, 1989.

Bessey PQ, Watters JM, Aoki TT et al. Combined hormonal infusionstimulates the metabolic response to thermal injury. Ann Surg 200: 264, 1984.

Beutler B. Cachexia: a fundamental mechanism. Nutr Rev 46: 369-373, 1988.

Bialick R, Gill R, Berke G et al. Modulation of cell-mediated cytotoxicity function after alteration of fatty acid composition in vitro. J Immunol 132: 81-87, 1984.

Birke G, Carlson LA, Liljedahl SO. Lipid metabolism and trauma. 3. Plasma lipids and lipoproteins in burns. Acta Med Scand 178: 337-350, 1965.

Bistrian B, Blackburn GL, Scrimshaw NS et al. Cellular immunity in semistarved states in hospitalized adults. Am J Clin Nutr 28: 1148-1155, 1975.

Bjornson AB, Bjornson HS, Knippenberg RW et al. Temporal relationships among immunologic alterations in a guinea pig model of thermal injury. J Infect Dis 153: 1098-1107, 1986.

Bjornson AB, Knippenberg RW, Bjornson HS. Bactericidal defect of neutrophils in a guinea pig model of thermal injury is related to elevation of intracellular cyclic-3',5'-adenosine monophosphate. J Immunol 143: 2609-2616, 1989.

Bjornson AB, Somers SD, Knippenberg RW et al. Circulating factors contribute to elevation of intracellular cyclic- 3',5'-adenosine monophosphate and depression of superoxide anion production in polymorphonuclear leukocytes following thermal injury. J Leukoc Biol 52: 407-414, 1992.

Black PR, Brooks DC, Bessey PQ et al. Mechanisms of insulin resistance following injury. Ann Surg 196: 420, 1982.

Blackburn GL. Nutrition and inflammatory events: highly unsaturated fatty acids (omega-3 vs omega-6) in surgical injury. Proc Soc Exp Biol Med 200: 183-188, 1992.

Blackwell TS and Christman JW. Sepsis and cytokines: current status. Br J Anaesth 77: 110-117, 1996.

Blazar BA, Rodrick ML, O'Mahony JB et al. Suppression of natural killer-cell function in humans following thermal and traumatic injury. J Clin Immunol 6: 26-36, 1986.

Blok WL, Katan MB, van der Meer JW. Modulation of inflammation and cytokine production by dietary (n- 3) fatty acids. J Nutr 126: 1515-1533, 1996.

Blok, W. L., Vogels, M. T., Curfs, J. H., Elling, W. M., Buurman, W. A., and van der Meer, J. W. Dietary fish oil supplementation in experimental gram-negative infection and in cerebral malaria in mice. J Infect Dis 165:898-903, 1992

Body JJ. The syndrome of anorexia-cachexia. Curr Opin Oncol 11: 255-260, 1999.

Boehm KD, Yun JK, Strohl KP et al. Messenger RNAs for the multifunctional cytokines interleukin-1 alpha, interleukin-1 beta and tumor necrosis factor-alpha are present in adnexal tissues and in dermis of normal human skin. Exp Dermatol 4: 335-341, 1995.

Boxer LA, Allen JM, Schmidt M et al. Inhibition of polymorphonuclear leukocyte adherence by prostacyclin. J Lab Clin Med 95: 672-678, 1980.

Braga M, Vignali A, Gianotti L et al. Immune and nutritional effects of early enteral nutrition after major abdominal operations. Eur J Surg 162: 105-112, 1996.

Brandtzaeg P, Halstensen K, Kett K et al. Immunobiology and immunopathology of human gut mucosa: Humoral immunity and intraepithelial lymphocytes. Gastroenterology 97: 1584, 1989.

Braquet M, Lavaud P, Dormont D et al. Leukocytic functions in burn-injured patients. Prostaglandins 29: 747-764, 1985.

Brass EP and Garrity MJ. Effect of E-series prostaglandins on cyclic AMP-dependent and -independent hormone-stimulated glycogenolysis in hepatocytes. Diabetes 34: 291-294, 1985.

Brass EP, Garrity MJ, Robertson RP. Inhibition of glucagon-stimulated hepatic glycogenolysis by E-series prostaglandins. FEBS Letters 169: 293-296, 1984.

Bray R, Abrams S, Brahmi Z. Studies on the mechanism of human natural killer cell-mediated cytolysis. I. Modulation by dexamethasone and arachidonic acid. Cell Immunol 78: 100-113, 1983.

Brenner R. Nutritional and hormonal factors influencing desaturation of essential fatty acids. Prog Lipid Res 20: 41-47, 1981.

Brittenden J, Heys SD, Ross J et al. Natural killer cells and cancer. Cancer 77: 1226-1243, 1996.

Brivio-Haugland RP, Louis SL, Musch K et al. Liver plasma membranes from essential fatty acid deficient rats. Isolation, fatty acid composition, and the activities of 5-nucleotidase, ATPase, and adenylate cyclase. Biochim Biophys Acta 433: 150-163, 1976.

Brom J, Koller M, Schluter B et al. Expression of the adhesion molecule CD11b and polymerization of actin by polymorphonuclear granulocytes of patients endangered by sepsis. Burns 21: 427-431, 1995.

Brown ER and Subbaiah PV. Differential effects of eicosapentaenoic acid and docosahexaenoic acid on human skin fibroblasts. Lipids 29: 825-829, 1994.

Bruera E, Carraro S, Roca E, et al. Association between malnutrition and caloric intake, emesis, psychological depression, glucose taste and tumor mass. Canc.Treat.Rep. 68:873, 1984

Bumpous JM and Johnson JT. The infected wound and its management. Otolaryngol Clin North Am 28: 987-1001, 1995.

Burch RM and Tiffany CW. Tumor necrosis factor causes amplification of arachidonic acid metabolism in response to interleukin 1, bradykinin, and other agonists. J Cell Physiol 141: 85-89, 1989.

Burleson DG, Mason ADJ, Pruitt BAJ. Lymphoid subpopulation changes after thermal injury and thermal injury with infection in an experimental model. Ann Surg 207: 208-212, 1988.

Burleson DG, Vaughn GK, Mason ADJ et al. Flow cytometric measurement of rat lymphocyte subpopulations after burn injury and burn injury with infection. Arch Surg 122: 216-220, 1987.

Burr, G. O. and Burr, M. M. A new deficiency disease produced by the rigid exclusion of fat from the diet. J Biol Chem 82:345-367, 1930

Caffrey BB and Jonsson HTJr. Role of essential fatty acids in cutaneous wound healing in rats. Prog Lip Res 20: 641-647, 1981.

Calder, P., Bond, J. A., Harvey, D. J., Gordon, S., and Newsholme, E. A. Uptake and incorporation of saturated and unsaturated fatty acids into macrophage lipids and their effect upon macrophage adhesion and phagocytosis. Biochem J 267:807-814, 1990

Calder PC. Fatty acids, dietary lipids and lymphocyte functions. Biochem Soc Trans 23: 302-309, 1995.

Calder PC, Bevan SJ, Newsholme EA. The inhibition of T-lymphocyte proliferation by fatty acids is via an eicosanoid-independent mechanism. Immunol 75: 108-115, 1992.

Calder PC, Yaqoob P, Newsholme EA. Triacylglycerol metabolism by lymphocytes and the effect of triacylglycerols on lymphocyte proliferation. Biochem J 298 Pt 3: 605-611, 1994.

Cantor H, Crump AL, Raman VK et al. Immunoregulatory effects of superantigens: interactions of staphylococcal enterotoxins with host MHC and non-MHC products. Immunol Rev 131: 27-42, 1993.

Carter EA and Tompkins RG. Injury-induced inhibition of fat absorption. J Burn Care Rehab 15: 154-157, 1994.

Cassatella MA, Gasperini S, Russo MP. Cytokine Expression and Release by Neutrophils. Ann N Y Acad Sci : 233-241, 2000.

Castell JV, Gomez-Lechon HJ, David M et al. Acute phase response of human hepatocytes regulation of acutep haseprotein synthesis by interleukin-6. Hepatol 12: 1179, 1990.

Caughey GE, Mantzioris E, Gibson RA et al. The effect on human tumor necrosis factor alpha and interleukin 1 beta production of diets enriched in n-3 fatty acids from vegetable oil or fish oil. Am J Clin Nutr 63: 116-122, 1996.

Cerra FB. Hypermetabolism, organ failure and metabolic support. Surg 101: 1-14, 1987.

Cerra FB, Lehmann S, Konstantinides N et al. Improvement in immune function in ICU patients by enteral nutrition supplemented with arginine, RNA, and menhaden oil is independent of nitrogen balance. Nutrition 7: 193-199, 1991.

Cerra FB, Siegel JH, Colman B et al. Septic autocannibalism, a failure of exogenous nutritional support. Ann Surg 192: 570-580, 1980.

Cetinkale O and Yazici Z. Early postburn fatty acid profile in burn patients. Burns 23: 392-399, 1997.

Chan SH, Kobayashi M, Santoli D et al. Mechanisms of IFN-gamma induction by natural killer cell stimulatory factor (NKSF/IL-12). Role of transcription and mRNA stability in the synergistic interaction between NKSF and IL-2. J Immunol 148: 92-98, 1992.

Chandra RK and Kumari S. Nutrition and immunity: an overview. J Nutr 124: 1433S-1435S, 1994.

Chandrasekar B and Fernandes G. Decreased pro-inflammatory cytokines and increased antioxidant enzyme gene expression by omega-3 lipids in murine lupus nephritis. Biochem Biophys Res Commun 200: 893-898, 1994.

Chang KJ, Saito H, Tatsuno I et al. Comparison of the effect of lipoxygenase metabolites of arachidonic acid and eicosapentaenoic acid on human natural killer cell cytotoxicity. Prost Leukot Essent Fatty Acids 38: 87-90, 1989.

Chapkin RS and Carmichael SL. Effects of dietary n-3 and n-6 polyunsaturated fatty acids on macrophage phospholipid classes and subclasses. Lipids 25: 827-834, 1990.

Chaudry A, McClinton S, Moffat LE et al. Essential fatty acid distribution in the plasma and tissue phospholipids of patients with benign and malignant prostatic disease. Br J Cancer 64: 1157-1160, 1991.

Chaux P, Favre N, Martin M et al. Tumor infiltrating dendritic cells are defective in their antigen presenting function and inducible B7 expression in rats. Int J Cancer 72: 619-625, 1997.

Chen X, Ogle CK, Wu JZ et al. The effect of thermal injury on biliary TNF, IL-6, PEG, and IgA. Proc Am Burn Ass 18: S74, 1997.

Cifone MG, Botti D, Festuccia C et al. Involvement of phospholipase A2 activation and arachidonic acid metabolism in the cytotoxic functions of rat NK cells. Cell Immunol 148: 247-258, 1993.

Clamp, A. G., Ladha, S., Clark, D. C., Grimble, R. F., and Lund, E. K. The influence of dietary lipids on the composition and membrane fluidity of rat hepatocyte plasma membrane. Lipids 32:179-84, 1997

Clandinin MT, Cheema S, Field CJ et al. Dietary fat: exogenous determination of membrane structure and cell function. FASEB J 5: 2761-2769, 1991.

Clandinin MT, Field CJ, Hargreaves K et al. Role of diet fat in subcellular structure and function. Can J Physiol Pharmacol 63: 546-556, 1985.

Cleary TG and Pickering LK. Mechanisms of intralipid effect on polymorphonuclear leukocytes. J Clin Lab Immunol 11: 21-26, 1983.

Cooper C. Nutrition and burns. 1997.

Corey, E. J., Shih, C., and Cashman, J. R. Docosahexaenoic acid is a strong inhibitor of prostaglandin but not leukotriene synthesis. Proc.Natl.Acad.Sci.USA 80:3581-3584, 1983

Cromack D, Porras-Reyes B, Purdy J et al. Acceleration of tissue repair by transforming growth factor: Identification of in vivo mechanisms of action with radiotherapy-induced specific healing deficits. Surg 113: 36-42, 1993.

Croset M, Bayon Y, Lagarde M. Incorporation and turnover of eicosapentaenoic and docosahexaenoic acids in human blood platelets in vitro. Biochem J 281: 309-316, 1992.

Cunningham-Rundles S and Lin DH. Nutrition and the immune system of the gut. Nutrition 14: 573-579, 1998.

Czaja AJ, Rizzo TA, Smith WRJ et al. Acute liver disease after cutaneous thermal injury. J Trauma 15: 887-894, 1975.

Czech MP and Fain JN. Antagonism of insulin action on glucose metabolism in white fat cells by dexamethasone. Endocrin 91: 518-522, 1972.

Dallegri F and Ottonello L. Neutrophil--mediated cytotoxicity against tumour cells: state of art. Arch Immunol Ther Exp (Warsz) 40: 39-42, 1992.

Daly JM, Redmond HP, Gallagher H. Perioperative nutrition in cancer patients. JPEN [illegible] Parenter Enteral Nutr 16: 100S-105S, 1992.

Daly JM, Reynolds J, Thom A. Immune and metabolic effects of arginine in the surgical patient. Ann Surg 209: 512-523, 1988.

Damtew B, Marino JA, Fratianne RB et al. Neutrophil lipoxygenase metabolism and adhesive function following acute thermal injury. J Lab Clin Med 121: 328-336, 1993.

Davis CF, Moore FDJ, Rodrick ML et al. Neutrophil activation after burn injury: contributions of the classic complement pathway and of endotoxin. Surg 102: 477-484, 1987.

Davis, J. M, Dineen, P., and Gallin, J. L. Neutrophil degranulation and abnormal chemotaxis after thermal injury. J.Immunol. 124:1467-1471, 1984

Davis JM, Dineen P, Gallin JI. Neutrophil degranulation and abnormal chemotaxis after thermal injury. J Immunol 124: 1467-1471, 1980.

De-Souza DA and Greene LJ. Pharmacological nutrition after burn injury. J Nutr 128: 797-803, 1998.

de Oliveira L, Borges MM, Leal RC et al. Nitric oxide involvement in experimental Trypanosoma cruzi infection in Calomys callosus and Swiss mice. Parasit Res 83: 762-770, 1997.

de Pablo MA and Alvarez de Cienfuegos G. Modulatory effects of dietary lipids on immune system functions. Immunol Cell Biol 78: 31-39, 2000.

de Pablo, M. A., Ortego, E., Gallego, A. M., Alverez, C., Pancorbo, P. L., and Alvarez de Cienfuegos, G. The effect of dietary fatty acid manipulation on phagocytic actvity and cytokine production by peritoneal macrophages from Balb/c mice. J.Nutr.Sci.Vitaminol. 44:67, 1998

Deitch EA. The relationship between thermal injury and neutrophil membrane functions as measured by chemotaxis, adherence and spreading. Burns Incl Therm Inj 10: 264-270, 1984.

Deitch EA and Landry KN. Neutrophil subpopulations change after thermal injury. J Trauma-Inj Infect Crit Care 26: 534-537, 1986.

Demichele SJ, Karlstad MD, Bistrian BR et al. Enteral nutrition with structured lipid: effect on protein metabolism in thermal injury. Am J Clin Nutr 50: 1295-1302, 1989.

Denham S and Rowland IJ. Inhibition of the reactive proliferation of lymphocytes by activated macrophages: the role of nitric oxide. Clin Exp Immunol 87: 157-162, 1992.

DeWys, W. D, Begg, C., Lavin, P., Band, PR, Bennett, J. M, Bertino, J. R., Cohen, M. H., and et al. Prognostic effect of weight loss prior to chemotherapy in cancer patients. Am.J.Med. 69:491-497, 1980

Dhanraj P, Chacko A, Mammen M et al. Hospital-made diet versus commercial supplement in postburn nutritional support. Burns 23: 512-514, 1997.

Dinarello CA. Biology of interleukin-1. FASEB J 2: 115, 1988.

Dinarello CA, Okusawa S, Gelfand JA. Prog Clin Biol Res 286: 243, 1989.

Dobke MK, Deitch EA, Harnar TJ et al. Oxidative activity of polymorphonuclear leukocytes after thermal injury. Arch Surg 124: 856-859, 1989.

Douglas RG and Shaw JH. Metabolic effects of cancer. Br.J.Surg. 77:246-254, 1990

Drost AC, Burleson DG, Cioffi WGJ et al. Plasma cytokines following thermal injury and their relationship with patient mortality, burn size, and time postburn. J Trauma 35: 335-339, 1993.

Duckett T and Belldegrun A. Immunology in cancer. Curr Opin Oncol 4: 149-155, 1992.

Duque RE, Phan SH, Hudson JL et al. Functional defects in phagocytic cells following thermal injury. Application of flow cytometric analysis. Am J Pathol 118: 116-127, 1985.

Dyerberg J, Bang HO, Aagaard O. Alpha-linolenic acid and eicosapentanoic acid. Lancet 1: 199, 1980.

Dyerberg, J., Bang, H. O., Stoffersen, G., Moncada, S., and Vane, J. R. Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis. Lancet 2:117-119, 1978

Efron JE, Frankel HL, Lazarou SA et al. Wound healing and T-lymphocytes. J Surg Res 48: 460-463, 1990.

Eicher SD and McVey DS. Dietary modulation of Kupffer cell and splenocyte function during a salmonella typhiurium challenge in mice. J Leukoc Biol 58: 32-39, 1995.

el-Falaky MH, Abdel-Hafez A, Houtah AH. Phagocytic activity of polymorphonuclear leucocytes in burns. Burns Incl Therm Inj 11: 185-191, 1985.

Elias JA, Gustilo K, Baeder W et al. Synergistic stimulation of fibroblast prostaglandin production by recombinant interleukin-1 and tumor necrosis factor. J Immunol 138: 3812-3816, 1987.

Endres S. N-3 polyunsaturated fatty acids and human cytokine synthesis. Lipids 31:S239-S242, 1996

Endres S, Ghorbani R, Kelley VE et al. The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. N Engl J Med 320: 265-271, 1989.

Falconer JS, Fearon DT, Ross JA et al. Acute phase protein response and survival duration of patients with pancreatic cancer. Cancer 75:2077-2082, 1995

Falconer, JS, Ross, JA, and Fearon, KCA phase II study of gamma-linolenic acid in pancreatic cancer and its effects on immune function and cytokine production In: New approaches to cancer treatment, Horrobin, D pp. 69.Longman,1994.

Farrar WL and Humes JL. The role of arachidonic acid metabolism in the activities of interleukin 1 and 2. J Immunol 135: 1153-1159, 1985.

Fernandes, G., Chandrasekar, B., Venkatraman, J., Tomar, V., and Zhao, W. Increased transforming growth factor beta and decreased oncogene expression by omega-3-fatty acids in the spleen delays the onset of autoimmune disease in B/W mice. J.Immunol. 152:5979-5987, 1994

Field CJ, Angel A, Clandinin MT. Relationship of diet to the fatty acid composition of human adipose tissue structural and stored lipids. Am J Clin Nutr 42: 1206-1220, 1985.

Field CJ, Toyomizu M, Clandinin MT. Relationship between dietary fat, adipocyte membrane composition and insulin binding in the rat. J Nutr 119: 1483-1489, 1989.

Fischer GW, Hunter KW, Wilson SR et al. Diminished bacterial defences with intralipid. Lancet 2: 819-820, 1980.

Fischer JE and Chance WT. Total parenteral nutrition, glutamine, and tumor growth. JPEN J Parenter Enteral Nutr 14: 86S-89S, 1990.

Fishel RS, Barbul A, Wasserkrug HL. Cyclosporin A impairs wound healing in rats. J Surg Res 34: 573-575, 1983.

Fleisher TA. Immune function. Pediatrics in Review 18: 351-356, 1997.

Flower RJ and Blackwell GJ. The importance of phospholipase-A2 in prostaglandin biosynthesis. Biochem Pharmacol 25: 285-291, 1976.

Fonteh AN, Samet JM, Surette M et al. Mechanisms that account for the selective release of arachidonic acid from intact cells by secretory phospholipase A2. Biochim Biophys Acta 1393: 253-266, 1998.

Franceschi S, Graham DL, Kim DH et al. Trauma alters the lymphocyte's plasma membrane fluidity. J Trauma 28: 1029, 1989.

Freid S, Appel B, Zechner R. Interleukin 1 alpha decreases the synthesis and activity of lipoprotein lipase in human adipose tissue. Horm Metab Res 25: 129-130, 1993.

Freid S and Zechner R. Cachetin/tumor necrosis factor decreased human adipose tissue lipoprotein lipase mRNA levels, synthesis and activity. J Lipid Res 30: 1917-1923, 1989.

Fujikawa M, Yamashita N, Yamazaki K et al. Eicosapentaenoic acid inhibits antigen-presenting cell function of murine splenocytes. Immunol 75: 330-335, 1992.

Fujishima S and Aikawa N. Neutrophil-mediated tissue injury and its modulation. Int Care Med 21: 277-285, 1995.

Fukushima R, Alexander JW, Wu JZ et al. Time course of production of cytokines and prostaglandin E2 by macrophages isolated after thermal injury and bacterial translocation. Circ Shock 42: 154-162, 1994.

Gadd MA and Hansbrough JF. The effect of thermal injury on murine neutrophil oxidative metabolism. J Burn Care Rehab 10: 125-130, 1989.

Gallin JI, Kaye D, O'Leary WM. Serum lipids in infection. N Engl J Med 281: 1081-1086, 1969.

Gallin JI, Wright DG, Malech HL et al. Disorders of phagocyte chemotaxis. Ann Intern Med 92: 520-538, 1980.

Gennari R and Alexander JW. Arginine, glutamine, and dehydroepiandrosterone reverse the immunosuppressive effect of prednisone during gut-derived sepsis. Crit Care Med 25: 1207-1214, 1997.

George S and Bugwarda N. Nutritional wound healing. Medsurg Nurs 5: 272-275, 1996.

Gerstein AD, Phillips TJ, Rogers GS et al. Wound healing and aging. Dermatol Clin 11: 749-757, 1993.

Gerster H. The use of n-3 PUFAs (fish oil) in enteral nutrition. Int J Vitam Nutr Res 65: 3-20, 1995.

Giacosa A, Frascio F, Sukkar SG et al. Food intake and body composition in cancer cachexia. Nutrition 12: S20-3, 1996.

Gibney MJ and Hunter B. The effects of short- and long-term supplementation with fish oil on the incorporation of n-3 polyunsaturated fatty acids into cells of the immune system in healthy volunteers. Eur J Clin Nutr 47: 255-259, 1993.

Gill R and Clark W. Effect of exogenously incorporated fatty acids on effector function in cell mediated cytolysis. J Immunol 125: 689-695, 1980.

Gogos CA, Ginopoulos P, Salsa B et al. Dietary omega-3 polyunsaturated fatty acids plus vitamin E restore immunodeficiency and prolong survival for severely ill patients with generalized malignancy: a randomized control trial. Cancer 82: 395-402, 1998.

Gogos CA, Kalfarentzos FE, Zoumbos NC. Effect of different types of total parenteral nutrition on T- lymphocyte subpopulations and NK cells. Am J Clin Nutr 51: 119-122, 1990.

Goodson WH and Hunt TK. Wound collagen accumulation in obese hyperglycaemic mice. Diabetes 35: 491-495, 1986.

Goodwin TS. Prostaglandins and Immunity. 1985. Boston, Martinus Nijoff.

Goppelt-Struebe M, Koerner CF, Hausmann et al. Control of prostanoid synthesis: role of reincorporation of released precursor fatty acids. Prostaglandins 32: 373-385, 1986.

Gottschlich MM. Selection of optimal lipid sources in enteral and parenteral nutrition. Nutr Clin Pract 7: 152-165, 1992.

Gottschlich MM and Alexander JW. Fat kinetics and recommended dietary intake in burns. JPEN J Parenter Enteral Nutr 11: 80-85, 1987.

Goust JM and Bierer B. Cell-mediated immunity. Immunol Ser 58: 187-212, 1993.

Graber R, Sumida C, Nunex, EA. Fatty acids and signal transduction. J.Lip.Med.Cell Signal. 9:91-116, 1994

Grayson LS, Hansbrough JF, Zapata-Sirvent RL et al. Quantitation of cytokine levels in skin graft donor site wound fluid. Burns 19: 401-405, 1993.

Grbic JT, Mannick JA, Gough DB et al. The role of prostaglandin E2 in immune suppression following injury. Ann Surg 214: 253-263, 1991.

Grimble RF and Tappia PS. The modulatory influence of unsaturated fatty acid in the biology of tumour necrosis factor. Biochem Soc Trans 287: 282-286, 1995.

Griswold JA. White blood cell response to burn injury. Semin Nephrol 13: 409-415, 1993.

Groff JL, Gropper SS, Hunt SM. Lipids In: Advanced Nutrition and Human Metabolism, 2, Purrington, L pp. 126-130.West Publishing Company, New York ,1995.

Gross RL and Newberne PM. Role of nutrition in immunologic function. Physiol Rev 60: 188-302, 1980.

Grunfeld C and Feingold KR. The metabolic effects of tumor necrosis factor and other cytokines. Biotherapy 3: 143-158, 1991.

Guillou PJ. The effects of lipids on some aspects of the cellular immune response. Proc Nutr Soc 52: 91-100, 1993.

Gurr MI. Role of fats in Food and Nutrition. 1992. London, UK, Elselvier Applied Science.

Gyllenhammar H, Hafstrom I, Borgeat P et al. Dietary linoleate supplementation modulates formyl-peptide receptor expression and functional responses of rat neutrophils. J Lab Clin Med 115: 487-496, 1990.

Hagve TA. Effects of unsaturated fatty acids on cell membrane functions. Scand J Clin Lab Invest 48: 381-388, 1988.

Hansbrough JF and Gadd MA. Temporal analysis of murine lymphocyte subpopulations by monoclonal antibodies and dual-color flow cytometry after burn and nonburn injury. Surg 106: 69-80, 1989.

Hansbrough JF, Wikstrom T, Braide M et al. Neutrophil activation and tissue neutrophil sequestration in a rat model of thermal injury. J Surg Res 61: 17-22, 1996.

Hansell DT, Davies JW, Burns HJG. The relationship between resting energy expenditure and weight loss in benign and malignant disease. Ann.Surg. 203:240-245, 1986

Hardardottir I, Doerrler W, Feingold KR et al. Cytokines stimulate lipolysis and decrease lipoprotein lipase activity in cultured fat cells by a prostaglandin independent mechanism. Biochem Biophys Res Commun 186: 237-243, 1992b.

Hardardottir I and Kinsella JE. Tumor necrosis factor production by murine resident peritoneal macrophages is enhanced by dietary n-3 polyunsaturated fatty acids. Biochim Biophys Acta 1095: 187-195, 1991.

Hardardottir I and Kinsella JE. Increasing the dietary (n-3) to (n-6) polyunsaturated fatty acid ratio increases tumor necrosis factor production by murine resident peritoneal macrophages without an effect on elicited peritoneal macrophages. J Nutr 122: 1942-1951, 1992a.

Harding FA, McArthur JG, Gross JA. CD28 mediated signalling costimulates murine T cells and prevents induction of anergy in T cell clones. Nature 356: 607-609, 1992.

Harris RL, Cottam GL, Johnston JM et al. The pathogenesis of abnormal erythrocyte morphology in burns. J Trauma 21: 13-21, 1981.

Harris RL, Frenkel RA, Cottam GL et al. Lipid mobilization and metabolism after thermal trauma. J Trauma 22: 194-198, 1982.

Hauner H, Petruschke T, Russ M et al. Effects of tumour necrosis factor alpha (TNF alpha) on glucose transport and lipid metabolism of newly-differentiated human fat cells in cell culture. Diabetologia 38: 764-771, 1995.

Head JF, Wang F, Elliott RL et al. Assessment of immunologic competence and host reactivity against tumor antigens in breast cancer patients. Prognostic value and rationale of immunotherapy development. Ann N Y Acad Sci 690: 340-342, 1993.

Hebert JC, O'Reilly M, Bednar MM. Modifying the host response to injury. The future of trauma care. Surg Clin North Am 75: 335-349, 1995.

Heck EL, Brown L, Curreri PW et al. Evaluation of leukocyte function in burned individuals by in vitro oxygen consumption. J Trauma 15: 486-489, 1975.

Heck EL, Edgar MA, Hunt JL et al. A comparison of leukocyte function and burn mortality. J Trauma 20: 75-77, 1980.

Heggers JP, Ko F, Robson MC et al. Evaluation of burn blister fluid. Plast Reconstr Surg 65: 798-804, 1980.

Heideman M and Hugli TE. Anaphylatoxin generation in multisystem organ failure. J Trauma-Inj Infect Crit Care 24: 1038-1043, 1984.

Heine J, Scheinichen D, Jaeger K et al. In vitro influence of parenteral lipid emulsions on the respiratory burst of neutrophils. Nutrition 15: 540-545, 1999.

Heinrich PC, Castell JV, Andus T. Interleukin-6 and the acute phase response. Biochem J 265: 621-636, 1990.

Herberman RB and Ortaldo JR. Natural killer cells: Their role in defenses against disease. Science 214:24-30, 1981

Herndon DN, Stein MD, Rutan TC et al. Failure of TPN supplementation to improve liver function, immunity, and mortality in thermally injured patients. J Trauma-Inj Infect Crit Care 27: 195-204, 1987.

Herschman HR. Function and regulation of prostaglandin synthase 2. Adv Exp Med Biol 469: 3-8, 1999.

Heuttner C, Paulus W, Roggendorf W. Messenger RNA expression of the immunosuppressive cytokine IL-10 in human gliomas. Am J Pathol 146: 317-322, 1995.

Heys SD, Gough DB, Khan L et al. Nutritional pharmacology and malignant disease: a therapeutic modality in patients with cancer. Br J Surg 83: 608-619, 1996.

Holt DR, Kirk SJ, Regan MC et al. Effect of age on wound healing in healthy human beings. Surg 112: 293-298, 1992.

Horrobin D. Nutritonal and medical importance of gamma-linolenic acid. Prog Lip Res 31: 163-194, 1992.

Huang M, Shamas S, Mao JT et al. Non-small cell lung cancer-derived soluble mediators and prostaglandin E2 enhance peripheral blood lymphocyte IL-10 transcription and protein production. J Immunol 157: 5812-5820, 1996.

Huang YS, Yang ZC, Liu XS et al. Serial experimental and clinical studies on the pathogenesis of multiple organ dysfunction syndrome (MODS) in severe burns. Burns 24: 706-716, 1998.

Hubbard NE, Lim D, Somers SD et al. Effects of in vitro exposure to arachidonic acid on TNF-alpha production by murine peritoneal macrophages. J Leukoc Biol 54: 105-110, 1993.

Hughes D and Pinder AC. n-3 polyunsaturated fatty acids inhibit the antigen-presenting function of human monocytes. Am J Clin Nutr 71: 357S-360S, 2000.

Hughes DA, Pinder AC, Piper Z et al. N-3 polyunsaturated fatty acids (PUFA) modulate the expression of functionally associated molecules on human monocytes. Biochem Soc Trans 23: 303S, 1995.

Hulsewe KW, van Acker BA, von Meyenfeldt MF et al. Nutritional depletion and dietary manipulation: effects on the immune response. World J Surg 23: 536-544, 1999.

Hundt M and Schmidt RE. The glycosylphosphatidylinositol-linked Fc gamma receptor III represents the dominant receptor structure for immune complex activation of neutrophils. Eur J Immunol 22: 811-816, 1992.

Hussey HJ and Tisdale MJ. Inhibition of tumour growth by lipoxygenase inhibitors. Br J Cancer 74: 683-687, 1996.

Imesch E and Rous S. Effect of PGE1 on gluconeogenesis and glycerol esterification in perfused liver of fasted rats. Prostaglandins 9: 945-957, 1975.

Ireton-Jones CS and Baxter CR. Nutrition for adult burn patients: a review. Nutr Clin Pract 6: 3-7, 1991.

Ireton-Jones CS and Turner WWJ. The use of respiratory quotient to determine the efficacy of nutrition support regimens. J Am Diet Assoc 87: 180-183, 1987.

Jacob TD, Ochoa JB, Udekwu AO et al. Nitric oxide production is inhibited in trauma patients. J Trauma-Inj Infect Crit Care 35: 590-596, 1993.

Jahoor F and Wolfe RR. Role of insulin and glucagon in the response of glucose and alanine kinetics in burn injured patients. J Clin Invest 78: 807, 1986.

Jarstrand C and Rasool O. Intralipid decreases the bacterial lipopolysaccharide induced release of oxygen radicals and lysozyme from human neutrophils. Scand J Infect Dis 23: 481-487, 1991.

Jeffery, N. M., Sanderson, P., Newsholme, P., and Calder, P. C. Characteristics of lipid and lymphocytes collected from the lymph of rats fed a low fat diet or high fat diets rich in n-6 or n-3 polyunsaturated fatty acids. Nutr.Res. 18:299-308, 1999

Jeffery NM, Sanderson P, Sherrington EJ et al. The ratio of n-6 to n-3 polyunsaturated fatty acids in the rat diet alters serum lipid levels and lymphocyte functions. Lipids 31: 737-745, 1996.

Jiang WG, Bryce RP, Horrobin DF. Essential fatty acids: molecular and cellular basis of their anti-cancer action and clinical implications. Crit Rev Onc Hem 27: 179-209, 1998.

Johannisson A and Festin R. Phenotype transition of CD4+ T cells from CD45RA to CD45R0 is accompanied by cell activation and proliferation. Cytometry 19: 343-352, 1995.

Johnson MM, Swan DD, Surette ME et al. Dietary supplementation with gamma-linolenic acid alters fatty acid content and eicosanoid production in healthy humans. J Nutr 127: 1435-1444, 1997.

Johnston PV. Dietary fat, eicosanoids, and immunity. Adv Lipid Res 21: 103-141, 1985.

Jondal M. The human NK cell:a short overview and an hypothesis on NK recognition. Clin.Exp.Immunol. 70:255-262, 1987

Jones PJ, Pencharz PB, Clandinin MT. Whole body oxidation of dietary fatty acids: implications for energy utilization. Am J Clin Nutr 42: 769-777, 1985.

June CH, Bluestone JA, Nadler LM et al. The B7 and CD28 receptor families. Immunol Today 15: 321-331, 1994.

Karlstad MD, Demichele SJ, Leathem WD et al. Effect of intravenous lipid emulsions enriched with gamma- linolenic acid on plasma n-6 fatty acids and prostaglandin biosynthesis after burn and endotoxin injury in rats. Crit Care Med 21: 1740-1749, 1993.

Kawakami M, Ishibashi S, Ogawa H et al. Biochem Biophys Res Commun 141: 482-487, 1986.

Kelley DS, Taylor PC, Nelson GJ et al. Arachidonic acid supplementation enhances synthesis of eicosanoids without suppressing immune functions in young healthy men. Lipids 33: 125-130, 1998.

Kelley DS, Taylor PC, Nelson GJ et al. Docosahexaenoic acid ingestion inhibits natural killer cell activity and production of inflammatory mediators in young healthy men. Lipids 34: 317-324, 1999.

Kelly JL, Lyons A, Soberg CC et al. Anti-interleukin-10 antibody restores burn-induced defects in T- cell function. Surg 122: 146-152, 1997.

Kern PA. Recombinant human tumor necrosis factor does not inhibit lipoprotein lipase in primary cultures of isolated human adipocytes. J Lipid Res 29: 909-914, 1988.

Keusch GT. Nutrition and immunity: from A to Z. Nutr Rev 56: S3-S4, 1998.

Kinsella JE. Lipids, membrane receptors, and enzymes: effects of dietary fatty acids. JPEN J Parenter Enteral Nutr 14: 200S-217S, 1990.

Kinsella JE and Lokesh B. Dietary lipids, eicosanoids, and the immune system. Crit Care Med 18: S94-113, 1990a.

Kinsella JE, Lokesh B, Broughton S et al. Dietary polyunsaturated fatty acids and eicosanoids: potential effects on the modulation of inflammatory and immune cells: an overview. Nutr 6: 24-62, 1990b.

Kitada S, Hays E, Mead JF et al. Lipolysis induction in adipocytes by a protein from tumor cells. J.Cell.Biochem. 20:409-416, 1982

Klein E and Mantovani A. Action of natural killer cells and macrophages in cancer. Curr Opin Immunol 5: 714-718, 1993.

Knudsen PJ, Dinarello CA, Strom TB. Prostaglandins posttranscriptionally inhibit monocyte expression of interleukin 1 activity by increasing intracellular cyclic adenosine monophosphate. J Immunol 137: 3189-3194, 1986.

Konig W, Koller M, Brom J. Priming mechanisms and induction of heat shock proteins in human polymorphonuclear granulocytes induced by eicosanoids and cytokines. Eicosanoids 5 Suppl: S39-41, 1992.

Konstantinides NN and Lehmann S. The impact of nutrition on wound healing. Crit Care Nurse 13: 25-33, 1993.

Kremer JM, Lawrence DA, Jubiz W et al. Dietary fish oil and olive oil supplementation in patients with rheumatoid arthritis. Arthritis Rheum 33: 810-820, 1990.

Kudsk KA, Croce MA, Fabian TC et al. Enteral versus parenteral feeding. Effects on septic morbidity after blunt and penetrating abdominal trauma. Ann Surg 215: 503-11; discussion 511-3, 1992.

Kumar S and Das U. Effect of prostaglandins and their precursors on the proliferation of human lymphocytes and their secretion of TNF and various interleukins. Prost Leukot Essent Fatty Acids : 331-334, 1994.

Kunkel SL, Chensue SW, Phan SH. Prostaglandins as endogenous mediators of interleukin 1 production. J Immunol 136: 186-192, 1986.

Kupper TS. Mechanisms of cutaneous inflammation. Interactions between epidermal cytokines, adhesion molecules, and leukocytes. Arch Dermatol 125: 1406-1412, 1989a.

Kupper TS. Production of cytokines by epithelial tissues. A new model for cutaneous inflammation. Am J Dermatopathol 11: 69-73, 1989b.

Kuschnaroff LM, Goebels J, Valckx D et al. Increased mortality and impaired clonal deletion after staphylococcal enterotoxin B injection in old mice: relation to cytokines and nitric oxide production. Scand J Immunol 46: 469-478, 1997.

Lambert B and Jacquemin C. Synergic effect of insulin and prostaglandin E1 on stimulated lipolysis. Prostagland Med 5: 375-382, 1980.

Last-Barnet K, Homom CA, Faanes RB et al. Synergistic and overlapping activities of tumor necrosis factor-alpha and IL-1. J.Immunol. 141:527-530, 1988

Lawrence, WT. Clinical management of nonhealing wounds In: Wound Healing: Biochemical and Clinical Aspects, Cohen, J, Deigelmann, R F, and Lindblad, W J pp. 541-561.WB Saunders Company, Philadelphia ,1992.

Lee TH, Hoover RL, Williams JD et al. Effect of dietary enrichment with eicosapentaenoic and docosahexaenoic acids on in vitro neutrophil and monocyte leukotriene generation and neutrophil function. N Engl J Med 312: 1217-1224, 1985.

Lefkowith JB, Jakschik BA, Stahl P et al. Metabolic and functional alterations in macrophages induced by essential fatty acid deficiency. J Biol Chem 262: 6668-6675, 1987.

Leger CL, Daveloose D, Christon R et al. Evidence for a structurally specific role of essential polyunsaturated fatty acids depending on their peculiar double bond distribution in biomembranes. Biochemistry 29: 7269-7275, 1990.

Leibovich SJ and Ross R. The role of the macrophage in wound repair. A study with hydrocortisone and antimacrophage serum. Am J Pathol 78: 71-100, 1975.

Leighton B, Budohoski L, Lozeman FJ et al. The effect of prostaglandins E1, E2 and F2 alpha and indomethacin on the sensitivity of glycolysis and glycogen synthesis to insulin in stripped soleus muscles of the rat. Biochem J 227: 337-340, 1985.

Lengle EE, Gustin NC, Gonzalez F et al. Energy metabolism in thymic lymphocytes of normal and leukemia AKR mice. Cancer Res 38: 1113-1119, 1978.

Lennard TW, Shenton BK, Borzotta A et al. The influence of surgical operations on components of the human immune system. Br J Surg 72: 771-776, 1985.

Lennartz MR. Phospholipases and phagocytosis: the role of phospholipid-derived second messengers in phagocytosis. Int J Biochem Cell Biol 31: 415-430, 1999.

Lester DS. In vitro linoleic acid activation of protein kinase C. Biochim Biophys Acta 1054: 297-303, 1990.

Letterio JJ and Roberts AB. Regulation of immune responses by TGF-beta. Annu Rev Immunol 16: 137-161, 1998.

Levitz SM and Dupont MP. Phenotypic and functional characterization of human lymphocytes activated by interleukin-2 to directly inhibit growth of Cryptococcus neoformans in vitro. J Clin Invest 91: 1490-1498, 1993.

Linscheer, WG and Vergroesen, AJLipids In: Modern Nutrition in Health and Disease, Shils, M E and Young, V R.Lea and Febiger, Philidelphia ,1988.

Linsley PS and Ledbetter JA. The role of CD28 receptor during T cell responses to antigen. Ann Rev Immunol 11: 191-212, 1993.

Liu XS, Luo ZH, Yang ZC et al. Clinical significance of the alterations of plasma prostaglandin E2 (PGE2) in severely burned patients. Burns 22: 298-302, 1996.

Llovera M, Garcia-Martinez C, Agell N et al. TNF can directly induce the expression of ubiquitin-dependent proteolytic system in rat soleus muscles. Biochem Biophys Res Commun 230: 238-244, 1997.

Locniskar M, Nauss KM, Newberne PM. The effect of quality and quantity of dietary fat on the immune system. J Nutr 113: 951-961, 1983.

Lokesh B, German B, and Kinsella JE. Differential effects of docosahexaenoic acid and eicosapentaenoic acid on suppression of lipoxygenase pathways in peritoneal macrophages. Biochim.Biophys.Acta 958:99-107, 1988

Lokesh BR and Wrann M. Incorporation of palmitic acid or oleic acid into macrophage membrane lipids exerts differential effects on the function of normal mouse peritoneal macrophages. Biochim Biophys Acta 792: 141-148, 1984.

Long CL, Crosby F, Geiger JW et al. Parenteral nutrition in the septic patient: nitrogen balance, limiting plasma amino acids, and calorie to nitrogen ratios. Am J Clin Nutr 29: 380-391, 1976.

Long CL, Jeevanandam M, Kinney J. Whole body protein synthesis and catabolism in septic man. Am J Clin Nutr 30: 1340-1344, 1977.

Lundholm K, Gelin J, Hyltander A et al. Anti-inflammatory treatment may prolong survival in undernourished patients with metastatic solid tumours. Cancer Res 54: 5602-5606, 1994.

Lyons A, Kelly JL, Rodrick ML et al. Major injury induces increased production of interleukin-10 by cells of the immune system with a negative impact on resistance to infection. Ann Surg 226: 450-468, 1997.

Marino JA, Gerding RL, Fratianne RB et al. Neutrophil adhesive dysfunction n thermal injury: the role of fibronectin. Infect.Dis. 157:674-681, 1988

Markovic SN and Murasko DM. Anesthesia inhibits interferon-induced natural killer cell cytotoxicity via induction of CD8+ suppressor cells. Cell Immunol 151: 474-480, 1993.

Marnett LJ. Polycyclic aromatic hydrocarbon oxidation during prostaglandin biosynthesis. Life Sci 29: 531-546, 1981.

Matsue H, Cruz PDJ, Bergstresser PR et al. Cytokine expression by epidermal cell subpopulations. J Invest Dermatol 99: 42S-45S, 1992.

McClinton S, Moffat LE, Horrobin DF et al. Abnormalities of essential fatty acid distribution in the plasma phospholipids of patients with bladder cancer. Br J Cancer 63: 314-316, 1991.

McGeer AJ, Detsky AS, O'Rourke K. Parenteral nutrition in cancer patients undergoing chemotherapy: a meta- analysis. Nutrition 6: 233-240, 1990.

McIrvine A, O'Mahony JB, Saporoschetz I et al. Depressed immune response in burn patients: use of monoclonal antibodies and functional assays to define the role of suppressor cells. Ann Surg 196: 297-304, 1982.

McManus AT. Examination of neutrophil function in a rat model of decreased host resistance following burn trauma. Rev Infect Dis 5 Suppl 5: S898-S907, 1983.

Melamed MR. Flow Cytometry and Sorting. Melamed, M. R., Lindmo, T., and Mendelsohn, M. L. 1990. New York, Wiley-Liss.

Memon A, Feingold KR, Moser A et al. In vivo effects of interferon alpha and interferon gamma on lipolysis and ketogenesis. Endocrin. 131:1695-1702, 1992

Meydani SN, Endres S, Woods MM et al. Oral (n-3) fatty acid supplementation suppresses cytokine production and lymphocyte proliferation: comparison between young and older women. J Nutr 121: 547-555, 1991.

Meyer NA, Muller MJ, Herndon DN. Nutrient support of the healing wound. New Horiz 2: 202-214, 1994.

Miller-Graziano CL, Fink M, Wu JY et al. Mechanisms of altered monocyte prostaglandin E2 production in severely injured patients. Arch Surg 123: 293-299, 1988.

Mittendorfer B, Jeschke MG, Wolf SE et al. Nutritional hepatic steatosis and mortality after burn injury in rats. Clin Nutr 17: 293-299, 1998.

Mizel SB. The interleukins. FASEB Journal 3: 2379-2388, 1989.

Molloy RG, O'Riordain D, Holzheimer R et al. Mechanism of increased tumor necrosis factor production after thermal injury. J Immunol 151: 2142-2149, 1993.

Monge G, Sparkes BG, Allgower M et al. Influence of burn-induced lipid-protein complex on IL1 secretion by PBMC in vitro. Burns 17: 269-275, 1991.

Montegut WJ and Lowry SF. Nutrition in burn patients. Semin Nephrol 13: 400-408, 1993.

Moore EE and Jones TN. Benefits of immediate jejunostomy feeding after major abdominal trauma-- a prospective, randomized study. J Trauma 26: 874-881, 1986.

Moore EE and Moore FA. Immediate enteral nutrition following multisystem trauma: a decade perspective. J Am Coll Nutr 10: 633-648, 1991.

Moore FA, Feliciano DV, Andrassy RJ et al. Early enteral feeding, compared with parenteral, reduces postoperative septic complications. The results of a meta-analysis. Ann Surg 216: 172-183, 1992.

Morain WD and Colen LB. Wound healing in diabetes mellitus. Clin Plast Surg 17: 493-501, 1990.

Moran KT, Allo MM, O'Reilly TJ et al. Neutrophil intracellular kill following thermal injury. Different bactericidal capability for patients' organisms and laboratory organisms. Arch Surg 123: 686-688, 1988.

Mosmann TR and Sad S. The expanding universe of T-cell subsets: Th1, Th2 and more. Immunol Today 17: 138-146, 1996.

Murphy JW, Hidore MR, Wong SC. Direct interactions of human lymphocytes with the yeast-like organism, Cryptococcus neoformans. J Clin Invest 91: 1553-1566, 1993.

Mustoe TA, Purdy J, Gamates P et al. Reversal of impaired wound healing in irradiated rats by platelet derived growth factor beta. Am J Surg 1598: 345-350, 1989.

Mutsaers D and Laurent G. Wound healing: from entropy to integrins. Int J Biochem Cell Biol : 1998.

Needleman, P., Raz, A., Minkes, M. S., Ferrendelli, J. A., and Sprecher, H. Triene prostaglandins: prostacyclin and thromboxane biosynthesis and unique biological properties. Proc.Natl.Acad.Sci.USA 76:944-948, 1979

Nelson RD, Hasslen SR, Ahrenholz DH et al. Mechanisms of loss of human neutrophil chemotaxis following thermal injury. J Burn Care Rehab 8: 496-502, 1987.

Nickoloff BJ, Turka LA, Mitra RS et al. Direct and indirect control of T-cell activation by keratinocytes. J Invest Dermatol 105: 25S-29S, 1995.

Nordenstrom J, Jarstrand C, Wiernik A. Decreased chemotactic and random migration of leukocytes during Intralipid infusion. Am J Clin Nutr 32: 2416-2422, 1979.

O'Connell J, Bennett MW, O'Sullivan GC et al. The fas-counterattack: cancer as a site of immune priviledge. Immunol Today 20: 46-52, 1999.

O'Mahony JB, Palder SB, Wood JJ et al. Depression of cellular immunity after multiple trauma in the absence of sepsis. J Trauma 24: 869-875, 1984.

O'Mahony JB, Wood JJ, Rodrick ML et al. Changes in T lymphocyte subsets following injury. Assessment by flow cytometry and relationship to sepsis. Ann Surg 202: 580-586, 1985.

O'Riordain MG, Collins KH, Pilz M et al. Modulation of macrophage hyperactivity improves survival in a burn- sepsis model. Arch Surg 127: 152-158, 1992.

O'Sullivan ST, Lederer JA, Horgan AF et al. Major injury leads to predominance of the T helper-2 lymphocyte phenotype and diminished interleukin-12 production associated with decreased resistance to infection. Ann Surg 222: 482-492, 1995.

O'Sullivan ST and O'Connor TP. Immunosuppression following thermal injury: the pathogenesis of immunodysfunction. Br J Plast Surg 50: 615-623, 1997.

Ogle CK, Guo X, Wu JZ et al. Production of cytokines and PGE2 and cytotoxicity of stimulated bone marrow macrophages after thermal injury and cytotoxicity of stimulated U-937 macrophages. Inflamm 17: 583-594, 1993.

Ogle CK, Mao JX, Wu JZ et al. The 1994 Lindberg Award. The production of tumor necrosis factor, interleukin-1, interleukin-6, and prostaglandin E2 by isolated enterocytes and gut macrophages: effect of lipopolysaccharide and thermal injury. J Burn Care Rehab 15: 470-477, 1994.

Ogle CK, Tchervenkov J, Alexander JW et al. The effect of high lipid diet on in vitro prostaglandin E2 and thromboxane B2 production by splenic macrophages. JPEN J Parenter Enteral Nutr 14: 250-254, 1990.

Okusawa S, Gelfand JA, Ikejima T et al. Interleukin-1 induces a shock-like sate in rabbits. Synergism with tumor necrosis factor and the effect of cyclooxygenase inhibition. J Clin Invest 81: 1162-1172, 1988.

Organ BC, Antonacci AC, Chiao J et al. Changes in lymphocyte number and phenotype in seven lymphoid compartments after thermal injury. Ann Surg 210: 78-89, 1989.

Osborne CK, Coronado EB, Robinson JP. Human breast cancer in the athymic nude mouse:cytostatic effect of long term anti-estrogen therapy. Eur J Canc Clin Onc 23: 1189, 1987.

Overath P, Schairer HU, Stoffel W. Correlation of in vivo and in vitro phase transitions of membrane lipids in Escherichia coli. Proc Natl Acad Sci USA 67: 606-612, 1970.

Ovesen L, Allingstrup L, Hannibal J et al. Effect of dietary counselling on food intake, body weight, response rate, survival ad quality of life in cancer patients undergoing chemotherapy: A prospective, randomized study. J Clin Onc 11: 2043-2049, 1993b.

Ovesen L, Hannibal J, and Mortensen EL. The interrelationship of weight loss, dietary intake and quality of life in ambulatory patiettns with cancer of the lung, breast and ovary. Nutr.Cancer 19:159-167, 1993a

Oxholm P, Pedersen BK, Horrobin DF. Natural killer cell functions are related to the cell membrane composition of essential fatty acids: differences in healthy persons and patients with primary Sjogren's syndrome. Clin Exp Rheumatol 10: 229-234, 1992.

Palombo JD, Demichele SJ, Lydon EE et al. Rapid modulation of lung and liver macrophage phospholipid fatty acids in endotoxemic rats by continuous enteral feeding with n-3 and gamma- linolenic fatty acids. Am J Clin Nutr 63: 208-219, 1996.

Palombo JD, Lydon E, Imrich A et al. The respiratory burst in alveolar macrophages is attenuated after displacement of phospholipid arachidonic acid by n-3 fatty acids in vivo. Surg Forum 46: 316-318, 1995.

Parker DC. T cell-dependent B cell activation. Ann Rev Immunol 11: 331-360, 1993.

Pascal de Bandt J, Chollet-Martin S, Hernvann A. Cytokine response to burn injury. J Trauma 36: 624-628, 1994.

Patten JA. Nutrition and wound healing. Compend Contin Educ Dent 16: 202-208, 1995.

Pawson, T. Growth factors, oncogenes and breast cancer In: Fundamental Problems in Breast Cancer, Paterson, A H G and Lees, A W pp. 155-171.Martinus Nijhoff, Boston ,1987.

Payan DG, Wong MY, Chernov-Rogan T et al. Alterations in human leukocyte function induced by ingestion of eicosapentaenoic acid. J Clin Immunol 6: 402-410, 1986.

Peck MD. Interaction of lipids with immune function I. Biochemical effects of dietary lipids on plasma membranes. J Nutr Biochem 5: 466-478, 1994b.

Peck MD. Omega-3 polyunsaturated fatty acids: benefit or harm during sepsis? New Horiz 2: 230-236, 1994a.

Peck MD. Nutritional Support for Burn injuries. J Nutr Biochem 10: 380-396, 1999.

Peck MD and Alexander JW. Survival in septic guinea pigs is influenced by vitamin E, but not by vitamin C in enteral diets. JPEN J Parenter Enteral Nutr 15: 433-436, 1991.

Pejnovic N, Lilic D, Zunic G et al. Aberrant levels of cytokines within the healing wound after burn injury. Arch Surg 130: 999-1006, 1995.

Penneys VS, Simon P, Ziboh VA et al. In vivo chemotaxis induced by polyunsaturated fatty acids. J Invest Dermatol 65: 435-438, 1977.

Peterson JM, Barbul A, Breslin RJ et al. Significance of T lymphocytes in wound healing. Surg 102: 300-305, 1987.

Pollack R, Lotzova E, Stanford S. Mechanism of surgical stress impairment on human perioperative natural killer cell cytotoxicity. Arch Surg 126: 338-342, 1991.

Poon R, Richards JM, Clark WR. The realtionship between plasma membrane lipid composition and physical-chemical properties. II. Effect of phospholipid fatty acid modification on plasma membrane physical properties and enzymatic activities. Biochim Biophys Acta 649: 58-66, 1981.

Pratt VC, Johnson IR, Field CJ. Glutamine and Arginine: Immunonutrients for improved health. Med Sci Sports Exerc 32: S377-S388, 2000.

Raina N, Matsui J, Cunnane SC et al. Effect of tumor necrosis factor-alpha on triglyceride and phospholipid content and fatty acid composition of liver and carcass in rats. Lipids 30: 713-718, 1995.

Ramesha CS and Ives DL. Detection of arachidonoyl-selective phospholipase A2 in human neutrophil cytosol. Biochim Biophys Acta 1168: 37-44, 1993.

Rappaport NH and Dodge GR. Prostaglandin E inhibits the production of interleukin-2. J Exp Med 155: 943-948, 1982.

Ras GJ, Eftychis HA, Anderson R et al. Mononuclear and polymorphonuclear leucocyte dysfunction in male homosexuals with the acquired immunodeficiency syndrome (AIDS). S Afric Med J 66: 806-809, 1984.

Richelsen B. Release and effects of prostaglandins in adipose tissue. Prost Leukot Essent Fatty Acids 47: 171-182, 1992.

Richelsen B and Pedersen O. Beta-adrenergic regulation of prostaglandin E2 receptors in human and rat adipocytes. Endocrin 116: 1182-1188, 1985.

Robin AP, Arain I, Phuangsab A et al. Intravenous fat emulsion acutely suppresses neutrophil chemiluminescence. JPEN J Parenter Enteral Nutr 13: 608-613, 1989.

Robinson LE and Field CJ. Dietary long-chain (n-3) fatty acids facilitate immune cell activation in sedentary, but not exercise-trained rats. J Nutr 128: 498-504, 1998.

Rodrick MI, O'Mahony JB, McIrvine A et al. Comparison of fluorescent microscopic and flow cytometric analyses of T cell subsets in burn patients. Fed Proc 42: 950, 1983.

Rodriquez JL, Miller CG, Garner WL et al. Correlation of the local and systemic cytokine response with clinical outcome following burn injury. J Trauma 34: 684-695, 1993.

Rola-Pleszczynski M. Immunoregulation by leukotrienes and other lipoxygenase metabolites. Immunol Today 6: 302-307, 1985.

Romagnani S, Manetti R, Annunziato F et al. Role of IL12 in the development of human Th1 type cells. Res Immunol 146: 452-460, 1995.

Roof RW, Luescher IF, Unanue ER. Phospholipids enhance the binding of peptides to class II major histocompatibility molecules. Proc Natl Acad Sci USA 87: 1735-1739, 1990.

Roper RL and Phipps RP. Prostaglandin E2 regulation of the immune response. Adv Prost Thrombox Leukot Res 22: 101-111, 1994.

Rosenberg CS. Wound healing in the patient with diabetes mellitus. Nurs Clin North Am 25: 247-261, 1990.

Rosenthal J, Thurman GW, Cusack N et al. Neutrophils from patients after burn injury express a deficiency of the oxidase components p47-phox and p67-phox. Blood 88: 4321-4329, 1996.

Rothe G and Valet G. Flow cytometric assays of oxidative burst activity in phagocytes. Methods Enzymol 233: 539-548, 1994.

Russell JK, Torres BA, Johnson HM. Phospholipase A2 treatment of lymphocytes provides helper signal for interferon-gamma production: Evidence for second messenger role of endogenous arachidonic acid. J Immunol 139: 3442-3446, 1987.

Saba TM and Luzio NR. Reticuloendothelial blockade and recovery as a function of opsonic activity. Am J Physiol 216: 197-205, 1979.

Sakata A, Ida E, Tominaga M et al. Arachidonic acid acts as an intracellular activator of the NADPH oxidase in Fcgamma receptor mediated superoxide generation in macrophages. J Immunol 138: 4353-4359, 1987.

Salmon M, Pilling D, Borthwick NJ et al. The progressive differentiation of primed T cells is associated with an increasing susceptibility to apoptosis. Eur J Immunol 24: 892-899, 1994.

Salo M. Inhibition of immunoglobulin synthesis in vitro by intravenous lipid emulsion (Intralipid). JPEN J Parenter Enteral Nutr 14: 459-462, 1990.

Sammon AM. Dietary linoleic acid, immune inhibition and disease. Postgrad Med J 75: 129-132, 1999.

Samuelsson B. Leukotrienes: mediators of immediate hypersensitivity reactions and inflammation. Science 220: 568-575, 1983.

Sassen LM, Lamers JM, Sluiter W et al. Development and regression of atherosclerosis in pigs. Effects of n-3 fatty acids, their incorporation into plasma and aortic plaque lipids, and granulocyte function. Arterioscler Thromb 13: 651-660, 1993.

Sayeed MM. Alterations in cell signaling and related effector functions in T lymphocytes in burn/trauma/septic injuries. Shock 5: 157-166, 1996.

Schroit AJ and Gallily R. Macrophage fatty acid composition and phagocytosis: effect of unsaturation on cellular phagocytic activity. Immunol 36: 199-205, 1979.

Scott, H. R., McMillan, D. C., Crilly, A., McArdle, C. S., and Milroy, R. The relationship between weight loss and interleukine 6 in non-small-cells lung cancer. Br.J.Cancer 73:1560-1562, 1996

Scrimshaw NS and SanGiovanni JP. Synergism of nutrition, infection, and immunity: an overview. Am J Clin Nutr 66: 464S-477S, 1997.

Seaman WE and Woodcock J. Human and murine natural killer cell activity may require lipoxygenation of arachidonic acid. J Allergy Clin Immunol 74: 407-411, 1984.

Seder, RA and Gazzinelli, RTCytokines are Critical in linking the Innate and adpative Immune responses to bacterial, fungal, and parasitic infection In: Advances in Internal Medicine pp. 353-359.Mosby, Inc.,1999.

Senkal M, Mumme A, Eickhoff U et al. Early postoperative enteral immunonutrition: clinical outcome and cost- comparison analysis in surgical patients. Crit Care Med 25: 1489-1496, 1997.

Sewell HF, Halbert CF, Robins RA et al. Chemotherapy-induced differential changes in lymphocyte subsets and natural-killer-cell function in patients with advanced breast cancer. Int J Cancer 55: 735-738, 1993.

Shangraw RE, Jahoor F, Miyoshi H et al. Differentiation between septic and post burn insulin resistance. Metabol 38: 983, 1989.

Shapiro AC, Wu D, Meydani SN. Eicosanoids derived from arachidonic and eicosapentaenoic acids inhibit T cell proliferative response. Prostaglandins 45: 229-240, 1993.

Shaw JH, Wildbore M, Wolfe RR. Whole body protein kinetics in severely septic patients. The response to glucose infusion and total parenteral nutrition. Ann Surg 205: 288-294, 1987a.

Shaw JH and Wolfe RR. Response to glucose and lipid infusions in sepsis: a kinetic analysis. Metabol 34: 442-449, 1985.

Shaw JH and Wolfe RR. Energy and protein metabolism in sepsis and trauma. Aust N Z J Surg 57: 41-47, 1987b.

Shaw JH and Wolfe RR. An integrated analysis of glucose, fat, and protein metabolism in severely traumatized patients. Studies in the basal state and the response to total parenteral nutrition. Ann Surg 209: 63-72, 1989.

Sheldon, H. Boyd's Introduction to the Study of Disease pp. 107-127.Philadelphia, Lea and Febiger,1992.

Sheridan RL, Yu YM, Prelack K et al. Maximal parenteral glucose oxidation in hypermetabolic young children: a stable isotope study. JPEN J Parenter Enteral Nutr 22: 212-216, 1998.

Sherry B and Cerami A. Cachectin/tumor necrosis factor exerts endocrine, paracrine, and autocrine control of inflammatory responses. J Cell Biol 107: 1269-1277, 1988.

Shike M, McRussel RD, Detsky AS et al. Changes in body composition in patients with small-cell lung cancer. Ann Intern Med 101:303, 1984

Shirani KZ, Vaughan GM, Mason ADJ et al. Update on current therapeutic approaches in burns. Shock 5: 4-16, 1996.

Shizgal HM. Body composition of patients with malnutrition and cancer. Cancer 55:250, 1985

Shou J, Lappin J, Minnard EA et al. Total parenteral nutrition, bacterial translocation, and host immune function. Am J Surg 167: 145-150, 1994.

Singer SJ and Nicolson MA. The fluid mosaic modle of the structure of the cell membrane. Science 175:730-831, 1972

Singh H, Abdullah A, Herndon DN. Effects of rat interleukin-2 and rat interferon on the natural killer cell activity of rat spleen cells after thermal injury. J Burn Care Rehab 13: 617-622, 1992.

Smith DJJ, Thomson PD, Garner WL et al. Donor site repair. Am J Surg 167: 49S-51S, 1994.

Smith EL, Hill RL, Lehmann IR et al. Lipid Metabolism I In: Triacylglycerols in principles of biochemistry General Aspects, 7, Smith, E L, Hill, R L, Lehmann, I R et. al. pp. 508-544.McGraw-Hill Book Company, New York ,1983.

Snijdewint FG, Kalinski P, Wierenga EA et al. Prostaglandin E2 differentially modulates cytokine secretion profiles of human T helper lymphocytes. J Immunol 150: 5321-5329, 1993.

Sobrado J, Moldawer LL, Pomposelli JJ et al. Lipid emulsions and reticuloendothelial system function in healthy and burned guinea pigs. Am J Clin Nutr 42: 855-863, 1985.

Solomkin JS. Neutrophil disorders in burn injury: complement, cytokines, and organ injury. J Trauma-Inj Infect Crit Care 30: S80-S85, 1990.

Solomon MJ and McLeod RS. Surgery and the randomised controlled trial: past, present and future. Med J Aust 169: 380-383, 1998.

Sosroseno W and Herminajeng E. The immunoregulatory roles of transforming growth factor beta. Br J Biomed Sci 52: 142-148, 1995.

Souba WW. Cytokine control of nutrition and metabolism in critical illness. Curr Probl Surg 31: 577-643, 1994.

Soyland E, Funk J, Rajka G et al. Dietary supplementation with very long-chain n-3 fatty acids in patients with atopic dermatitis. A double-blind, multicentre study. Br J Dermatol 130: 757-764, 1994.

Spagnuolo PJ, Ellner JJ, Hassid A et al. Thromboxane A2 mediates augmented polymorphonuclear leikocyte adhesiveness. J Clin Invest 66: 406-414, 1980.

Spector AA and Yorek MA. Membrane lipid composition and cellular function. J Lipid Res 26: 1015-1035, 1985.

Sriskandan S, Moyes D, Buttery LK et al. The role of nitric oxide in experimental murine sepsis due to pyrogenic exotoxin A-producing Streptococcus pyogenes. Infect Immun 65: 1767-1772, 1997.

Stoner HB. Energy metabolism after injury. J Clin Path - Suppl (Royal College of Pathologists) 4: 47-55, 1970.

Streat SJ and Hill GL. Nutritional support in the management of critically ill patients in surgical intensive care. World J Surg 11: 194-201, 1987.

Strunk RC, Kunke KS, Kolski GB et al. Intralipid alters macrophage membrane fatty acid composition and inhibits complement (C2) synthesis. Lipids 18: 493-500, 1983.

Stubbs CD and Smith AD. The modification of mammalian membrane polyunsaturated fatty acid composition to membrane fluidity and function. Biochim Biophys Acta 779: 89-137, 1984.

Sukkar SG, Giacosa A, and Frascio F. Clinical validation of bioelectrical impedence (BIA) in malnourished cancer patients. RINPE 11:78, 1993

Swain SL. Lymphokines and the immune response:the central role of Interleukin-2. Curr Opin Immunol 3: 304-310, 1991.

Szabo G, Kodys K, Miller-Graziano CL. Elevated monocyte interleukin-6 (IL-6) production in immunosuppressed trauma patients. I. Role of Fc gamma RI cross-linking stimulation. J Clin Immunol 11: 326-335, 1991.

Szamel M and Resch K. Modulation of enzyme activities in isolated lymphocyte plasma membranes by enzymatic modification of phospholipid fatty acids. J Biol Chem 256: 11618-11623, 1981.

Taylor DD, Gercel-Taylor C, Jenis L et al. Identification of a human tumor-derived lipolysis-promoting factor. Cancer Res 52:829-834, 1992

Telfer NR and Moy RL. Drug and nutrient aspects of wound healing. Dermatol Clin 11: 729-737, 1993.

Teodorczyk-Injeyan JA, McRitchie DI, Peters WJ et al. Expression and secretion of IL-2 receptor in trauma patients. Ann Surg 212: 202-208, 1990.

Teodorczyk-Injeyan JA, Sparkes BG, Mills GB et al. Impaired expression of interleukin-2 receptor (IL2R) in the immunosuppressed burned patient: reversal by exogenous IL2. J Trauma 27: 180-187, 1987.

Teodorczyk-Injeyan JA, Sparkes BG, Mills GB et al. Impairment of T cell activation in burn patients: a possible mechanism of thermal injury-induced immunosuppression. Clin Exp Immunol 65: 570-581, 1986.

Tisdale MJ. Inhibition of lipolysis and muscle protein degradation by EPA in cancer cachexia. Nutrition 12: S31-S33, 1996.

Tisdale MJ. Wasting in cancer. J Nutr 129: 243S-246S, 1999.

Tiwary R, Clandinin MT, Cinader B. Effect of high polyunsaturated fat diets on the compostion of B cell and T cell membrane lipids. Nutr Res 7: 489-498, 1987.

Todd JC and Mollitt DL. Erythrocyte membrane alterations induced by sepsis. J Trauma 31: 1040, 1991.

Todorov P, Cariuk P, McDevitt T et al. Characterisation of a cacner cachectic factor. Nature 379: 739-742, 1996.

Tolentino MV, Sarusua MM, Hill OA et al. Peripheral lymphocyte membrane fluidity after thermal injury. J Burn Care Rehab 12: 498-504, 1991.

Tomita-Yamaguchi M, Rubio C, Santoro TJ. Regional influences on the physical properties of T cell membranes. Life Sci 48: 433-438, 1991.

Torosian MH. Cachexia and Cancer. Canc J 8: 299-303, 1995.

Traill KN and Wick G. Lipids and Lymphocyte Function. Immunol Today 5: 20-76, 1986.

Tredget EE and Yu YM. The metabolic effects of thermal injury. World J Surg 16: 68-79, 1992.

Trinchieri G. Proinflammatory and immunoregulatory functions of interleukin-12. Int Rev Immunol 16: 365-396, 1998.

Tripp CS, Wolf SF, Unanue ER. Interleukin 12 and tumor necrosis factor alpha are costimulators of interferon gamma production by natural killer cells in severe combined immunodeficiency mice with listeriosis, and interleukin 10 is a physiologic antagonist. Proc Natl Acad Sci USA 90: 3725-3729, 1993.

Tsujinaka T, Fujita J, Ebisui C et al. Interleukine-6 receptor antibody inhibits muscle atrophy and modulates proteolytic system in interleukin-6 transgenic mice. J Clin Invest 97: 244-249, 1996.

Turek JJ, Schoenlein IA, Clark LK et al. Dietray polyunsaturated fatty acid effects on immune cells of the porcine lung. J Leukoc Biol 56: 599-604, 1994.

Van Den Hove L, Van Gool SW, Vandenberghe P et al. CD40 triggering of chronic lymphocytic leukemia B cells results in efficient alloantigen presentation and cytotoxic T-lymphocyte induction by upregulation of CD80 and CD86 costimulatory molecules. Leukemia 11: 572-580, 1997.

Van Gool SW, Van Den Hove L, Ceuppens JL. Activation of the immune system in cancer patients. Med Ped Onc 34: 1-9, 2000.

Van Way CW3. Nutritional support in the injured patient. Surg Clin North Am 71: 537-548, 1991.

Vega GL and Baxter CR. Induction of hypertriglyceridemia in rabbits by thermal injury: I. Time course of elevated plasma triglyceride levels. J Burn Care Rehab 9: 266-270, 1988.

Virella G, Fourspring K, Hyman B et al. Immunosuppressive effects of fish oil in normal human volunteers: correlation with the in vitro effects of eicosapentanoic acid on human lymphocytes. Clin Immunol Immunopathol 61: 161-176, 1991.

Virella G, Kilpatrick JM, Rugeles MT et al. Depression of humoral responses and phagocytic functions in vivo and in vitro by fish oil and eicosapentanoic acid. Clin Immunol Immunopathol 52: 257-270, 1989.

von Schacky C, Fisher S, Weber PC. Long-term effects of dietary marine n-3 fatty acids upon plasma and cellular lipids, platelet function, and eicosanoid formation in humans. J Clin Invest 76: 1626-1631, 1985.

Wakefield CH, Carey PD, Foulds S et al. Polymorphonuclear leukocyte activation. An early marker of the postsurgical sepsis response. Arch Surg 128: 390-395, 1993.

Wallace FA, Yaqoob P, Miles EA et al. Dietary fat influences the production of Th1- but not Th2-derived cytokines. Lipids 34 Suppl: S141, 1999.

Weaver, B. J. and Holub, B. J. The inhibition of arachidonic acid incorporation into human platlet phospholipids by eicosapentaenoic acid. Nutr.Res. 5:31-37, 1985

Wigmore SJ, Fearon KC, Maingay JP et al. Down-regulation of the acute-phase response in patients with pancreatic cancer cachexia receiving oral eicosapentaenoic acid is mediated via suppression of interleukin-6. Clin Sci 92: 215-221, 1997.

Wigmore SJ, Pleater CE, Richardson RA et al. Changes in nutritional status associated with unresectable pancreatic cancer. Br J Cancer 75: 106-109, 1997.

Wigmore SJ, Ross JA, Falconer JS et al. The effect of polyunsaturated fatty acids on the progress of cachexia in patients with pancreatic cancer. Nutrition 12: S27-S30, 1996.

Witte MB and Barbul A. General principles of wound healing. Surg Clin North Am 77: 509-528, 1997.

Wolfe RR. Herman Award Lecture, 1996: relation of metabolic studies to clinical nutrition--the example of burn injury. Am J Clin Nutr 64: 800-808, 1996.

Wolfe RR, Herndon DN, Jahoor F et al. Effect of severe burn injury on substrate cycling by glucose and fatty acids. N Engl J Med 317: 403-408, 1987.

Wolfe RR, Jahoor F, Hartl WH. Protein and amino acid metabolism after injury. Diabetes Metab Rev 5: 149-164, 1989.

Wolfe RR, Shaw JH, Jahoor F et al. Response to glucose infusion in humans: role of changes in insulin concentration. Am J Physiol 250: E306-11, 1986.

Wood JJ, Rodrick ML, O'Mahony JB et al. Inadequate interleukin 2 production. A fundamental immunological deficiency in patients with major burns. Ann Surg 200: 311-320, 1984.

Woodward B. Protein, calories, and immune defenses. Nutr Rev 56: S84-S92, 1998.

Wu D, Meydani SN, Meydani M et al. Immunologic effects of marine- and plant-derived n-3 polyunsaturated fatty acids in nonhuman primates. Am J Clin Nutr 63: 273-280, 1996.

Wu JZ, Ogle CK, Mao JX et al. The increased potential for the production of inflammatory cytokines by Kupffer cells and splenic macrophages eight days after thermal injury. Inflamm 19: 529-541, 1995.

Yamashita N, Sugiyama E, Hamazaki T et al. Inhibition of natural killer cell activity by eicosapentaenoic acid in vivo and in vitro. Biochem Biophys Res Commun 150: 497-505, 1988.

Yamashita N, Yokoyama A, Hamazaki T et al. Inhibition of natural killer cell activity of human lymphocytes by eicosapentaenoic acid. Biochem Biophys Res Commun 138: 1058-1067, 1986.

Yaqoob, P., Newsholme, E. A., and Calder, P. C. The effect of dietary lipid manipulation on rat lymphocyte subsets andproliferation. Immunol. 82:603-610, 1994

Yaqoob P, Newsholme EA, and Calder PC. Influence of cell culture conditions on diet-induced changes in lymphcoyte fatty acid composition. Biochim Biophys Acta 1255:333-340, 1995

Yeagle PL. Regulation of membrane function through composition, structure, and dynamics. Ann N Y Acad Sci 568: 29-34, 1989.

Yoshino S and Ellis EF. Effectof a fish oil-supplemented diet on inflammation and immunological processes in rats. Int Arch Allerg Immunol 84:233-240, 1987

Zapata-Sirvent RL and Hansbrough JF. Temporal analysis of human leucocyte surface antigen expression and neutrophil respiratory burst activity after thermal injury. Burns 19: 5-11, 1993.

Zechner R, Newman TC, Sherry B et al. Recombinant human cachectin/tumor necrosis factor but not interleukin-1 alpha downregulates lipoprotein lipase gene expression at the transcriptional level in mouse 3T3-L1 adipocytes. Mol Cell Biol 8: 2394-2401, 1988.

Zidek Z and Masek K. Erratic behavior of nitric oxide within the immune system: illustrative review of conflicting data and their immunopharmacological aspects. Int J Immunopharm 20: 319-343, 1998.

Zimmerli W, Waldvogel FA, Vaudaux P et al. Pathogenesis of foreign body infection: description and characteristics of an animal model. J Infect Dis 146: 487-497, 1982.

Zurier RB. Fatty acids, inflammation and immune responses. Prost Leukot Essent Fatty Acids 48: 57-62, 1993.

II.

Research Plan

A. Rationale

Critical illness results in a plethora of metabolic and immunologic abnormalities. A major component of patient care during critical illness is nutrition with the basic objectives being to provide nutrients for growth and repair of tissues, maintenance of body cell mass and prevention of infection (Gottschlich et al., 1987). Ideally, one would aim to provide the specific nutrients or combination of nutrients that are most immunologically and functionally beneficial in a specific group of patients. Clinical trials examining the effects of specific fatty acids to nutritional formulations designed for critically ill patients have been inconclusive (Section I.F.5; Klein et al., 1997). Many factors must be taken into account when determining the optimal fatty acid content and composition in the diet during disease states characterized by metabolic alterations and immunosuppression.

It is known that essential fatty acids have profound effects on metabolism and immune function. During stress states, there are perturbations in fatty acid metabolism (Gottschlich et al., 1987) that may alter fatty acid composition of phospholipids in membranes which impact on cellular function of immune cells. Recovery from critical illness is often complicated by the presence of infection. Infection has distinct effects on metabolism and immune function apart from the disease state that further suppresses immune function and depletes nutritional status (Alexander and Peck, 1990; Chandra and Kumari, 1994). Infection is due to a breakdown in host immune function, therefore, it is reasonable to postulate that improving immune parameters important in infection defense would decrease incidence of infections. Dietary fat can modulate PL composition and ultimately cellular function (Clandinin et al., 1991). N-3 fatty acids displace arachidonic acid and are known to suppress inflammatory responses and also enhance immune functions (Calder and de Cienfuegos,1995; de Pablo et al., 2000). Despite our knowledge that fatty acid composition of immune cells influences their function, changes in lipid composition of neutrophils and lymphocytes that occur following burn injury and during cancer have not been characterized previously. The immunological impairments that occur during cancer, burns and chemotherapy have not been examined in relation to membrane composition even though diet is an important part of the therapy. Therefore, characterization of alterations in lymphocyte proliferation and cytokine production in response to mitogens, NK cell cytotoxicity, neutrophil oxidative burst and phenotype changes that are associated with membrane phospholipid changes are warranted. Furthermore, the efficacy of intervention with specific fatty acids and the ability of the host to utilize them during critical illness needs to be examined before nutritional intervention strategies can be formulated. To understand the relationship between immune cell membrane composition and their function, it is necessary to measure properties and components of membrane lipids to extrapolate how cellular processes change in relation to diet and disease. Lipid sources in alternative nutrient formulations designed for specific patient populations must be chosen to promote membrane phospholipid composition and immune function that is consistent with recovery. By identifying specific changes in lipid metabolism and immune function as a result of these disease states, a practical intervention strategy can be developed with an objective measure to assess the efficacy of such formulations.

The overall objective of this thesis research is to demonstrate that **alterations in fatty acid composition of immune cells and plasma lipid components exist in disease states characterized by metabolic impairments and immunosuppression and contribute to functional changes observed in both the cell mediated and innate immune branches.**

B. Objectives and Hypotheses

1. Effects of Burn injury on Phospholipid Composition and Function of Immune Cells

Objective: To characterize changes in membrane PL fatty acid composition and immune function during recovery from thermal injury to establish the importance of membrane composition in immune recovery and infection defense.

Hypotheses: It is hypothesized that:

a) the phospholipid fatty acid composition of immune cells, namely lymphocytes and neutrophils, will be altered after burn injury and change in the direction of healthy individuals during recovery of the patient. More specifically:

(i) The phospholipids of lymphocytes and neutrophils will have a lower content of arachidonic acid immediately post-injury in the major phospholipid fractions and increase with recovery.

(ii) Total n-6 and n-3 fatty acids in the phospholipids of lymphocytes and neutrophils will be reduced in the early post burn period and will increase during recovery in the direction of that seen in the healthy population.

b) There will be impaired function of immune cells of both the acquired and innate immune branches post-burn injury. More specifically:

(i) After burn, the relative percentages of CD4 cells will be low.

(ii) Cell mediated immunity as estimated by mitogen stimulated ^{3}[H] thymidine incorporation will be decreased at 1-12 days post injury and increase as the patient recovers. Unstimulated [^{3}H] thymidine incorporation will be high initially and decrease with recovery.

(iii) The TH1 subset will be activated immediately post-injury with a high release of IFN-γ and IL-2.

(iv) T cells (CD4+ and CD8+) will express a high percentage of CD45RA (antigen naivity) in the initial post burn period and percent CD45RO (antigen mature) will increase by the second timepoint (d12-19). Activation markers (CD25 and CD71) on lymphocytes will be high early after burn and decrease with recovery.

(v) Natural Killer cell activity will be low initially following burn injury and increase with recovery.

(vi) Neutrophil activation will be high initially producing high amounts of oxygen radicals during the first weeks following burn. Unstimulated neutrophil activity will decrease as the patient recovers.

(vii) The proportion of macrophages (particularly activated macrophages) will be high at the first sample point post-burn injury.

2. The Effects of Burn Injury on Fatty Acid Composition of Plasma Components and Erythrocytes

Characterization of the fatty acid composition of plasma components is necessary to better understand the metabolic effects of burn injury on fatty acid metabolism. This is important in determining if the abnormalities observed in immune cell PL are specific to that tissue or is there is an overall impairment in the metabolism of essential fatty acids.

Objective: To characterize the fatty acid composition of plasma CE, TG and PL from early to late post burn timepoints.

Hypotheses: It is hypothesized that:

a) the 20:4n-6 content of plasma CE, TG and PL as well as RBCs will be reduced following burn injury and increase with recovery.

b) there will be reduced concentrations of CE and PL and elevated concentrations of TG in plasma early post burn that return to normal levels (those observed in healthy individuals) with recovery from injury.

3. The Effect of Surgery and Infection on Immune Function in Guinea Pigs

Surgery and infection are components of recovery after burn injury and cancer treatments that contribute to immunosuppression. The third objective of this research was to characterize changes in immune function associated with surgery and infection.

Objective: To determine the effect of surgery, and surgery + infection on measures of the specific and innate immune branches in lymphoid tissues of a guinea pig model of infection. The effect of surgery + infection + Acticoat™ Silver coated dressing on immune recovery and function will be measured.

Hypothesis: It is hypothesized in a guinea pig model that:

a) surgery will result in reductions in the oxidative burst activity of neutrophils, decreased natural killer cell cytotoxicity, reduced mitogen stimulated ^{3}[H]-thymidine uptake, and a reduced CD4/CD8 ratio in lymphoid tissues.

b) infection with *Staphylococcus aureus* post surgery will result in an increased oxidative burst of neutrophils, increased MHC Class II molecule expression on immune cells and a further (compared to the effects of surgery alone) depression of NK cytotoxicity and mitogen stimulated [^{3}H]-thymidine uptake.

c) infected animals treated with the Acticoat™ Silver coated dressing will have immune responses similar to the noninfected surgery animals.

4. The Effect of Stem Cell Harvest and High Dose Chemotherapy on Immune Function in Women With Breast Cancer

Dietary recommendations for women with breast cancer undergoing high dose chemotherapy with stem cell transplant (described in Chapter VII) has not been established. This treatment has severe consequences on nutritional status and immune function. However, nutritional intervention is not part of this treatment. Immune recovery in this group is a clinical problem induced by the treatment rather the disease. Little is known about the immune changes and essential fatty acid status of women who have received high dose chemotherapy with stem cell transplant.

Objective: to characterize fatty acid and immune changes at stem cell harvest and after reconstitution (described in Chapter VII) in women undergoing high dose chemotherapy followed by transplant of stem cells for breast cancer.

Hypothesis: It is hypothesized that:

a) At discharge, cellular function will be reduced from pre-HDCT levels. Specifically, compared to harvest values the following measures will be different at discharge:
   (i) The CD4/CD8 ratio will be reduced
   (ii) Neutrophil oxidative burst will be reduced
   (iii) T cells will have lower expression of activation markers
   (iv) The expression of the CD45RO isotype on T-lymphocytes (CD4+ and CD8+) will be decreased

b) Neutrophil cell membranes and plasma lipid components will suggest deficiencies in essential fatty acid metabolism both at harvest and at discharge. These deficiencies will be more pronounced following the high dose procedure.

5. The effects of Fish Oil supplementation on Immune Functions in Cachexic Cancer Patients

Cachexia is a complication of cancer. Nutritional intervention has been largely unsuccessful in altering cachexia. Recently, it has been reported that feeding n-3 fatty acids can reduce cachexic complications in cancer patients.

Objective: To determine the effect of supplementing the normal diet of palliative cancer patients with 18g of fish oil or placebo per day for 14 days on immune changes associated with infection, as well as the effects on fatty acid composition of immune cells. Fatty acid composition of plasma phospholipids will be characterized in patients supplemented with fish oil or a placebo oil (olive oil).

Hypothesis: It is hypothesized that:

a) Supplementing weight losing cancer patients with 18 g of long chain polyunsaturated fatty acids/d for 14 d, compared to a placebo will result in an increase in long chain n-3 fatty acids in the membranes of neutrophils as well as in the plasma fatty acids.

b) Supplementing weight losing cancer patients with 18 g of long chain polyunsaturated fatty acids/d for 14 d, compared to a placebo will improve some measures of immune functions in cells of peripheral blood (parameters of the acquired and innate immune system). Specifically:

(i) the CD4/CD8 ratio will increase.

(ii) the expression of CD45RO on CD4+ and CD8+ T cells will increase.

(iii) the expression of the IL-2 receptor and CD28 on immune cells will increase.

(iv) The neutrophil oxidative burst will decrease.

6. Changes in Phospholipid Composition of Neutrophils Impacts on Their Functional Capacity

There were deficiencies observed in essential fatty acid metabolism in each of the unique patient groups described. Changes in neutrophil function were most distinguishable and were thought to be most affected by changes in diet because many aspects of their function is dependent to some degree on membrane fatty acids. Experiments that associate immune function with alterations in specific membrane fatty acids are needed to demonstrate benefits of dietary intervention in each patient group.

Objective: to demonstrate a relationship between increases or decreases in the 20:4n-6 and n-3 fatty acid content of the membrane to functional changes in neutrophils using a primary cell culture model.

Hypothesis: It is hypothesized that:

a) Increasing the 20:4n-6 content in neutrophil PL will increase the oxidative burst of neutrophils.

b) Increasing the n3 fatty acid content of neutrophil PL will decrease the oxidative burst of neutrophils.

C. Chapter Layout

The hypothesis posed were tested in a sequence of experiments. These experiments are organized as thesis chapters and have been or will be submitted for scientific publication as individual papers.

Chapter 3 characterizes membrane PL fatty acid composition and the function of neutrophils during recovery from thermal injury. It establishes the importance of membrane composition in immune recovery and infection defense *(hypothesis 1.a and b (vii))*. This chapter entitled "**Changes in neutrophil fatty acid composition and its relationship to neutrophil function following burn injury in humans**" has been submitted to the Journal of Lipid Research for scientific review.

Chapter 4 examines the effects of burn injury on the PL composition of lymphocytes and characterizes alterations in proliferation, cytokine production, immune phenotypes and NK cytotoxicity associated with burn injury and recovery *(hypothesis 1a, b (i-iv))*. This chapter entitled "**Alterations in lymphocyte function and relation to PL composition following burn injury**" has been submitted to the journal Critical Care Medicine for scientific review.

Chapter 5 characterizes the changes observed in the essential fatty acid composition of plasma CE, PL and TG as well as RBCs from early to late post burn *(hypothesis 2)*. This chapter entitled "**Fatty acid content of plasma lipids and**

erythrocyte phospholipids are altered following burn injury" has been submitted to Lipids for scientific review.

Chapter 6 explores the effect of surgery, and surgery + infection on measures of the specific and innate immune branches in lymphoid tissues of a guinea pig model of infection *(hypothesis 3a, b)*. In addition, the effects of Acticoat™ on immune parameters was examined in an infected guinea pig model *(hypothesis 3c)*.

Chapter 7 is a pilot study that characterizes changes in fatty acids and immune parameters before and after high dose chemotherapy followed by stem cell transplant for breast cancer *(hypothesis 4)*.

Chapter 8 examines the immunological effects of supplementing cachexic cancer patients with fish oil or placebo for 14 days on T cell phenotypes and neutrophil function. In addition, plasma PL and immune cell fatty acid compositions are characterized both before and after supplementation with fish oil or placebo *(hypothesis 5)*. This work constitutes the pilot work for a clinical trial proposal.

Chapter 9 consists of *in vitro* primary cell culture work that demonstrates a relationship between arachidonic acid in neutrophil membranes and their functional abilities. This work has been submitted in conjunction with Chapter 3 in a manuscript entitled **"Changes in neutrophil fatty acid composition and its relationship to neutrophil function following burn injury in humans"** to the Journal of Lipid Research for scientific review.

Chapter 10 is an overall general summary and discussion of the potential relationships between membrane composition and immune function observed in diseases characterized by metabolic and immunological impairments.

D. References

Alexander JW and Peck MD. Future prospects for adjunctive therapy: pharmacologic and nutritional approaches to immune system modulation. Crit Care Med 18: S159-64, 1990.

Calder PC. Fatty acids, dietary lipids and lymphocyte functions. Biochem Soc Trans 23: 302-309, 1995.

Chandra RK and Kumari S. Nutrition and immunity: an overview. J Nutr 124: 1433S-1435S, 1994.

Clandinin MT, Cheema S, Field CJ, et al. Dietary fat: exogenous determination of membrane structure and cell function. FASEB J 5: 2761-2769, 1991.

de Pablo MA and Alvarez de Cienfuegos G. Modulatory effects of dietary lipids on immune system functions. Immunol Cell Biol 78: 31-39, 2000.

Gottschlich MM and Alexander JW. Fat kinetics and recommended dietary intake in burns. JPEN J Parenter Enteral Nutr 11: 80-85, 1987.

Klein S, Kinney J, Jeejeebhoy K, et al. Nutrition support in clinical practice: review of published data and recommendations for future research directions. National Institutes of Health, American Society for Parenteral and Enteral Nutrition, and American Society for Clinical Nutrition. JPEN J Parenter Enteral Nutr 21: 133-156, 1997.

III.

Changes in Neutrophil Fatty Acid Composition and its Relationship to Neutrophil Function Following Burn Injury in Humans.

A. Introduction

Until recently the most frequent cause of death in burn patients was sepsis and multiple organ failure (MOF; Arturson,1985). Infection remains an important threat and abnormalities in neutrophil function following burn injury have been suggested to contribute significantly to development of infection and risk of sepsis in these patients (Alexander et al.,1978; Alexander et al.,1979; Bjornson et al.,1992; Davis et al.,1980; Grogan et al.,1973). Defense mechanisms employed by neutrophils to fight infection can also be damaging to the host (Simms,1995). Reactive oxygen intermediates produced by activated neutrophils have been implicated in tissue damage characteristic of multiple organ dysfunction syndrome (Matzner,1997). Pulmonary failure, manifested by acute respiratory distress syndrome (ARDS) and pneumonia, is presently the major cause of death following burn injury (Hansbrough et al.,1996). The pathophysiology of ARDS is not entirely elucidated, however, it is accompanied by accumulation of neutrophils (Fujishima et al.,1995) followed by local release of toxic mediators and increased energy oxygen metabolism (Hansbrough et al., 1996) resulting in tissue injury that leads to organ dysfunction (Fujishima et al., 1995).

Lipids and their products of metabolism are known to significantly impact on immune function. Fatty acid metabolism is altered following burn injury and inflammatory mediators derived from lipids are increased. Phospholipase A_2 (PLA_2) activity increases (Rosenthal et al.,1995b) which results in release of arachidonic acid, a precursor for prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4), two important mediators involved in inflammation and neutrophil activation. The relationship between composition of phospholipid membranes and cellular immune function is well established (Bialick et al.,1984; Calder et al.,1991; Robinson et al.,1998). How compositional changes impact neutrophil function following burn injury is not known. Diet is an important adjunct in the treatment of burn injured

A version of this chapter has been submitted for publication.
Pratt et al., 2001. Journal of Lipid Research.

patients (Saffle et al.,1997) and dietary fat influences phospholipid composition of immune cells (Chapkin et al.,1990; Palombo et al.,1997). There is considerable controversy regarding the optimal amount and composition of fat that should be fed to these patients (Gerster,1995; Gottschlich et al.,1987; Peck,1994). Before dietary fat recommendations can be formulated to improve immune function in thermally injured patients, data is needed to characterize fatty acid metabolism post burn and understand how alterations in essential fatty acids contribute to changes in immune function. This study was designed to investigate the compositional changes in neutrophil phospholipid fatty acids and functional alterations immediately following and during recovery from burn injury.

B. Methods

1. Materials

RPMI 1640 culture media and all other culture ingredients were purchased from Fisher Scientific (Edmonton, AB, Canada). Dihydrorhodamine 123 (DHR) was purchased from Molecular Probes (Eugene, OR). Phorbol myristate acetate (PMA) was purchased from ICN (Montreal, PQ, Canada). H-plates were purchased from Analtech (Newark, DE) and solvents were purchased from VWR (Edmonton, AB, Canada). Ficoll hypaque gradients, bovine serum albumin (BSA, Fraction V), chemicals for buffers and all other lipid supplies, including standards, were purchased from Sigma Chemicals (St. Louis, MO).

2. Subjects

The study was approved by the University of Alberta Faculty of Medicine Research Ethics Board. Approval to obtain blood samples from healthy subjects was obtained from the Faculty of Agriculture, Forestry and Home Economics Research Ethics Board. Ten subjects were recruited from patients admitted to the Firefighter's Burn Treatment Unit at the University of Alberta Hospital (Edmonton, Alberta, Canada) after informed consent. Patients who had >10% total body surface area (TBSA) burn and who gave informed consent were included in the study. Patients who were under the age of 18, overtly malnourished, had a history of alcohol or drug abuse, were taking immunosuppressive drugs or had autoimmune disease were excluded. Table III.1 shows the characteristics of subjects enrolled. All subjects were previously healthy and survived their injuries. Their age range was from 20 to 65 years of age (mean=40 ± 4 years). The range of burn size was from 12-90% (mean=37 ± 5 % TBSA). Subjects were resuscitated in Ringer's lactate (Warden,1973) using the Parkland formula (Leape,1970). All patients underwent wound excision and skin grafting of deep second and third degree wounds commencing the first week of hospitalization. All subjects received similar wound care treatment, analgesia and antibiotic therapy using pre-established protocols. A non-fasting blood sample was drawn from healthy volunteer subjects (n=6) recruited from the University of Alberta who gave informed consent.

3. Dietary Intake

Energy requirements for each subject were determined using the Harris Benedict equation using a stress factor based on injury severity. Tube feeds were started within 24 hours post injury. The enteral diet fed provided high amounts of nitrogen and was specially designed for critical care patients (Nitro-Pro™, Nutrition Medical, Minneapolis, MN). The nutrient composition of Nitro-Pro™ is shown in Table III.2. The fat content of the enteral formula was composed of 50% medium chain triglycerides and 50% corn oil for a total of 29% of total energy coming from fat. The fatty acid composition of the formula is given in Table III.3. ProMod™ (Ross Laboratories, Columbus OH), a whey protein concentrate, was supplemented as an extra source of protein when required and its composition is given in Table III.4. The dietary intake of clinical feeds was recorded for each patient during the inpatient period. No patients in the study received total parenteral nutrition.

4. Sample Collection

A non fasting venous blood sample (10 ml) was collected by the medical staff during the patient's regular blood work on the following days after admission to the burn unit: within the first 12 days (t1), between 12 and 19 days (t2), between 20 and

35 days (t3), between 26 and 49 days (t4) and after 50 days (t5). For subjects with smaller burns or those who were discharged prior to 50 days, the final sample was obtained at the subject's first outpatient visit to the clinic. When more than one sample was obtained within a time period, the results were combined and the mean value reported. If blood sample scheduling coincided with surgery days, samples were taken prior to surgery to avoid short term confounding effects of blood transfusions and surgery on the immune response.

5. Hematological Analysis

Complete blood counts (CBC), using a Coulter STKS instrument (Coulter Electronics, Inc., Hialeah, FA), and manual differential were performed by the staff of the Hematology Laboratory at the University of Alberta Hospital.

6. Preparation of Neutrophils

Neutrophils were isolated using Ficoll Hypaque gradient centrifugation (Boyum,1968) by layering 3 mls of whole blood on top of 3 mls of each 1119 and 1077 (neutrophil and lymphocyte density gradients, respectively) and centrifuging at 1840 rpm for 30 minutes with no brake at room temperature. The neutrophil band was transferred into 15 ml conical tubes using a transfer pipette. Krebs-Ringer HEPES (KRH) buffer + BSA (5g/L) was added up to the top of the tube and sample was centrifuged (Beckman J2-HC, Beckman Instruments, Palo Alto, CA) for 10 minutes at 1000 rpm to pellet cells. If necessary, red blood cells in the fraction were lysed using warmed lysis buffer and cells washed twice more with KRH + 0.5% (w/vol) BSA. After the last wash, 1 ml of RPMI 1640 supplemented with fetal calf serum (50 g/L), HEPES (25 mmol/L), penicillin (100 units/mL) and streptomycin (100 μg/mL) was added to the samples and viability was assessed using trypan blue exclusion. Cell viability was greater than 99% for all samples.

7. Fatty Acid Analysis

a) *Lipid Extraction*

A modified Folch method (Folch et al.,1957) was used to extract lipids from gradient isolated neutrophils (Field,1988). After the addition of KCL, the suspension was transferred to a clean methylation tube. Methanol (0.8 ml), 2.0 ml chloroform/methanol (1:1 vol/vol), 2.7 ml chloroform and 2.5 ml chloroform/methanol (2:1 vol/vol) were added sequentially with vortexing after each step. Samples were capped, vortexed and kept at 4°C overnight to obtain good phase separation. The next day the bottom layer containing the phospholipids was removed to a clean methylation tube and dried down under nitrogen gas. Chloroform (1 ml) was added to the original tube, vortexed, and after phase separation, the bottom layer was removed and added to the drying down samples. Chloroform (100 μl) was added to the dried sample.

b) *Thin Layer Chromatography*

Samples in chloroform described above were spotted onto thin layer chromatography plates (HPK silica gel 60 Å 10 x 10 cm, Whatman, Clifton, NJ) that had been heat activated at 110°C for one hour. Solvent tanks were lined with Whatman #1 filter paper and saturated with the solvent systems for 1 hour before plates were placed in the tank. Samples were run in a chloroform/methanol/2-propanol/0.25% KCL/triethylamine (30:9:25:6:18 vol/vol) solvent system in a 13x13 cm chamber at room temperature for approximately 45 minutes or until sample reached the top (Touchstone et al.,1980). Plates were dried in a dessicator and separated phospholipids visualized with 8-anilino-1-naphthalene-sulfonic acid (ANSA, 0.1%, w/v) and identified under ultraviolet light using appropriate standards. Bands corresponding to phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), and phosphatidylserine (PS) were scraped and added to clean methylation tubes.

c) *Methylation*

Samples were methylated immediately using 0.75 ml 14% (w/w) boron trifluoride (BF_3) in methanol and 1.5 ml of distilled hexane. Methylation proceeded for 1 hour at 110°C in a sand bath. After samples cooled, 1 ml of dd H_2O was added, the sample vortexed and stored overnight at 4°C. The next day the upper phase was removed and added to a GC vial and dried down under nitrogen. An additional 0.5 ml of ddH_2O was added to the tube, vortexed and after separation was added to the GC vial. Dried samples were flushed with nitrogen, capped and held at -70°C until analysis.

d) *Gas Liquid Chromatography*

Samples were analyzed by automated gas liquid chromatography (Vista 6010, Varian Instruments, Georgetown, ON) on a fused silica BP20 capillary column (25 m x 0.25 mm internal diameter, Varian Instruments; (Field et al.,1989). Fatty acid methyl esters were separated by an automated gas-liquid chromatograph, varian model 6000 equipped with a vista 654 data system and a Vista 8000 autosampler (Varian Instrument Company, Georgetown Ontario). The system used a bonded phase fused silica capillary column, BP20: 25 mm x 0.25 OD SCG product. Helium was used as the carrier gas at a flow rate of 1.8 ml/minute using a splitless injector. The GLC oven temperature of 150°C was increased to 190°C at 20°C/minute and held for 23 minutes followed by a second stage temperature increase to 220°C at 2/minute for a total analysis time of 40 minutes. These conditions separate all saturated, monounsaturated and polyunsaturated fatty acids from 14 to 24 carbon chain lengths. Percent content of saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA), total n-6 and n-3 fatty acids and the 20:4n-6 content were analyzed at each timepoint post burn injury.

8. Neutrophil Functional Parameters

Whole blood (400 µl) was added to 4 mls warmed lysis buffer in sterile polypropylene tubes and gently inverted several times for 5 minutes. Cells were pelleted by centrifuging for 5 minutes at 1500 rpm, washed once with warmed wash buffer and again pelleted. Cells were reconstituted in 400 µl of wash buffer and 1.8 µl DHR (29 mM in DMSO) was added to each reaction tube and incubated in a water bath (37°C) for 5 minutes. An aliquot was removed from the tube (0 minutes) and PMA (3.2×10^3 nM) was added. Tubes were incubated for 5, 10 or 15 minutes more after which time an aliquot of cells was removed, transferred to an immunofluorescence tube and immediately placed on ice to stop the reaction (Vowells et al.,1995). The oxidation of dihydrorhodamine 123 to rhodamine 123 was immediately quantified by flow cytometry (FACScan, Becton Dickenson, San Jose, CA) using a 488 ηm line for excitation with collection occurring at 525 ηm. The data was analyzed using CellQuest software (Becton Dickenson). Neutrophils were discriminated from the remaining leukocytes, red blood cells and debris by combined measures of forward and side light scatter characteristics. Non-fluorescent parameters were collected (mean forward scatter and mean side scatter) to estimate changes in size and granularity (measures of activation) after 5, 10 and 15 minutes. Mean channel fluorescence of gated neutrophils was measured at 0, 5, 10 and 15 minutes and gives an estimation of the oxidative burst (See Figure I.8). The change in granularity, size and oxidative burst after stimulation was determined using a ratio given by the formula (5,10 or 15 minute value/0 minute value).

9. Statistical Analysis

Data is reported as mean ± SEM. To determine differences between different timepoints post burn injury, a repeated measures analysis of variance was used using the post burn time periods described above (<12 days=t1; 12 -19 days=t2; 20 - 35 days=t3, 26 - 49 days=t4; >50 days=t5). Subjects were grouped into groups based on size of burn (large= >35% TBSA, n=5 and small=<35% TBSA, n=5) and when differences existed between these two groups, results are presented. When there were no differences between groups, all subjects were included in the analysis and overall mean presented. Number of subjects included in the analysis at each timepoint was as follows: t1, n=10; t2, n=10; t3, n=8; t4, n=6; t5, n=8. Subjects with burns >35% TBSA were sampled at every timepoint. Significant differences ($p<0.05$) between time periods were identified using least square means. To determine if subjects followed the same pattern of changes in neutrophil function and phospholipid composition during recovery, orthoganol comparisons of the linear ($y=mx+b$; time after burn = x) and quadratic equations were used. All statistical analyses were conducted using the SAS statistical package (Version 6.12, SAS Institute, Cary, NC).

C. Results

1. Dietary Intake

Enteral feeds began 24 hours post burn injury and were generally well tolerated. Complications included diarrhea (2/10 subjects), emesis (2/10 subjects), and hyponatemia (1/10 subjects). Enteral feeds were stopped when feeding complications persisted and 6 hours prior to surgery, therefore values in Table III.1 include those periods of reduced intake and estimate average daily intake when Nitro-Pro™ provided the majority of the energy consumed. Subjects consumed an average of 9132 kJ (2183 kcal) per day (Table III.1) during the inpatient period when enteral feeds were the primary source of energy. The average intake of linoleic acid (18:2n-6) during the inpatient period was 21 ± 1 g/day (189 kcal/day; 9% energy) which approximates the intake of healthy, free-living North Americans who are estimated to consume 3-18 % of energy from linoleic acid (James et al.,1993). Mean linolenic acid (18:3n-3) consumption per day coming from this diet was 0.6 ± 0.02 g/day (Table III.1).

2. Neutrophil Concentration

There were significantly fewer neutrophils per ml blood at t5 than t1 ($2.4 \pm 0.4 \times 10^6$ vs $6.8 \pm 1.5 \times 10^6$; $p<0.04$) as determined by trypan blue and crystal violet staining. Total white blood cell counts from CBCs performed in hospital were not performed for all subjects at t4 and t5 due to discharge prior to these sampling times. For those measures done, white blood cell counts did not change significantly over time, however, the mean white blood cell count exceeded reference values ($4\text{-}11 \times 10^9$ cells/ml) at t1 ($12 \pm 1 \times 10^9$ cells/ml; n=8) and t2 ($15 \pm 1 \times 10^9$ cells/ml; n=8).

3. Neutrophil Functional Parameters

Freshly isolated neutrophils (time 0) were significantly larger at t1 than at t3, t4 and t5 ($p<0.03$, Table III.5). No significant differences were observed in the size ratio at any timepoint post burn (mean=1.04 ± 0.03; data not shown). Freshly isolated neutrophils (time 0) were significantly less granular at t2 than at t3 or t5 (Table III.6). Ten minutes post stimulation, the granularity ratio was significantly greater for neutrophils obtained at t4 and t5 than those obtained at t1 (Table III.6). At 15 minutes post stimulation, the granularity ratio at t5 was significantly greater than all other stimulation timepoints (Table III.6). The respiratory burst (mean fluorescence) at 10 minutes was significantly greater at t2 compared to t1 (Figure III.1) and compared to t3 and t5 ($p<0.02$, statistics not illustrated in Figure III.1).

4. Fatty Acid Composition of Neutrophils

a) 20:4n-6 and 18:2n-6

All major phospholipid fractions demonstrated significantly less total 20:4n-6 at t1 than at t5 (Figure III.2, Table III.7). Compared to t1, total 20:4n-6 content in the PC fraction significantly increased by t3 whereas the other 3 phospholipid fractions did not show a significant increase until t5 (Table III.7). The PC fraction had a significantly greater 18:2n-6/20:4n-6 ratio at t1 and t2 compared to t5 (Table III.8) but no significant changes in total 18:2n-6 content during the post burn period (Table III.10). The PS fraction contained significantly more 18:2n-6 at t1, t3 and t4 than at t5 (Table III.11) and a ten fold higher 18:2n-6/20:4n-6 ratio at t1, t2 and t3 than at t5 (Table III.8). There were no significant differences in 18:2n-6 content (Table III.12, Table III.10) or the 18:2n-6/20:4n-6 ratio in the PE or PI fractions (Table III.8) between post burn timepoints.

b) Total n-6, n-3 and n-6/n-3 ratio

There were significantly higher proportions of n-6 fatty acids at t5 than the first 3 timepoints in the PS fraction (Table III.9). The PI fraction contained a significantly greater proportion of n-6 fatty acids at t5 than t1 (Table III.10). There were no significant differences at any post burn timepoint in total n-6 content of PC and PE fractions when all subjects are included in the analysis. However, when subjects with the greatest % TBSA burns (>35%) were analyzed, there was a significant difference ($p<0.05$) in the n-6 content of PE between t1 (30.3 ± 2.6%; n=5) and t5 (40.5 ± 2.64%; n=5). There were no changes in the percent composition of individual major n-3 fatty acids (18:3n-3, 18:4n-3, 20:5n-3, 22:5n-3, 22:6n-3) in the PC fraction (Table III.9) during the post burn period, however, total n-3 content of PC was significantly greater at t5 than t1 and t2 (Table III.9) resulting in a significant difference in the n-6/n-3 ratio between t2 (9.6 ± 1.4) and t5 (6.6 ± 2.0) in the PC fraction. The n-6/n-3 ratio for the other phospholipid fractions did not change between post burn timepoints and the mean ratio was PS: 7.7 ± 0.8, PI: 17.5 ± 3.6 and PE: 6.0 ± 0.5. Total n-3 fatty acids of the other phospholipids fractions did not change significantly during the post burn timepoints, however, alterations in individual n-3 fatty acids during recovery post burn are shown in Tables III.10-12.

c) Total SFA, PUFA and MUFA

PI contained significantly higher proportions of SFA at t1 than t2 and t4 (Table III.10) and PE contained a significantly greater proportion of SFA at t3 and t4 than t5 (Table III.12). Generally, the proportion of total MUFA in each of the PL classes decreased during recovery. This trend was significant for PC whereby t1 contained significantly more MUFA than t4 and t5 (Table III.9) and for PE which contained significantly less MUFA at the last 3 timepoints compared to t1 and t2 (Table III.12). Total PUFA increased with recovery from burn injury in the PE, PC and PS fractions. Compared to t1, the PUFA content of PC increased significantly at t4 (Table III.19), whereas PS didn't increase significantly from t1 until t5 (Table III.11). The PE fraction had a significant increase in PUFA from t1 at t3 and then again at t5 making the PUFA content of neutrophils obtained at t5 greater ($p<0.05$) than the first 3 timepoints in the PE fraction (Table III.12). Detailed tables of the complete fatty acid composition of neutrophil PL fractions after burn injury and in healthy individuals are available in Appendices III.AP1-AP5.

D. Discussion

Dietary lipids alter fluidity and contribute to structural modifications of fatty acid components in membrane phospholipids. Major effector responses of neutrophils necessary for killing but also implicated in tissue injury include activation of the oxidative burst and exocytosis of primary granules (Simms,1995) both of which have been reported to be altered following burn injury (Braquet et al.,1985; Dobke et al.,1989; Duque et al.,1985; Gadd et al.,1989; Heck et al.,1980). This study demonstrates a relationship between the essential fatty acid composition of neutrophil phospholipids and their function following burn injury.

All major phospholipids of neutrophils had lower 20:4n-6 content early post burn that increased with recovery. Low 20:4n-6 levels in neutrophils coincided with increased stimulated oxidative burst and reduced granularity (t2). Reductions in 20:4n-6 content of neutrophils could be due to increased release, decreased synthesis or both. The PC fraction was the quickest to increase in 20:4n-6 content, returning to late recovery levels by the third week post burn whereas PS, PI and PE took longer for 20:4n-6 levels to significantly increase from immediate post burn levels. Release of 20:4n-6 from membranes is the rate limiting step for synthesis of bioactive lipid mediators (Mahadevappa et al.,1989; Rosenthal et al.,1995a), known to be increased post burn. Enzymatic cleavage of PI results in formation of IP_3 which increases intracellular calcium, a prerequisite for oxidative burst activity (Sayeed,1998) and degranulation (Smolen,1989). Some subjects in this study had 10 fold less 20:4n-6 content at t1 compared to late recovery timepoints in their PI fraction. Activated neutrophils have been reported to have increased PLA_2 activity (Jordan et al.,1999) which has also been reported to be involved in eliciting the oxidative burst response (Condliffe et al.,1998). Our observations provide support for increased release of 20:4n-6 from membranes in the early post burn period.

The fatty acid content of the enteral formula fed to these subjects ensured an adequate intake of 18:2n-6 (Diboune et al.,1992), thus the reductions in 20:4n-6 in neutrophil PL in the post burn period are likely the result of the burn injury rather than a dietary deficiency. Comparisons to other reports that have investigated changes in membrane compositions with different dietary intakes in this population and others is difficult due to the failure to measure individual PL. However, the 20:4n-6 levels we have found in neutrophil PL from healthy individuals are generally higher than those observed at the early post burn timepoints in this study (Appendix III.AP5). The subjects in this study received very little dietary n-3 fatty acids in their diet therefore, competitive inhibition by n-3 lipids was unlikely the cause of lower 20:4n-6 levels. Higher linoleic acid content observed in the PS fraction, increased 18:2n-6/20:4n-6 ratio in the PC and PI fractions observed in this study and reported elsewhere (Braquet et al., 1985) with a high intake of 18:2n-6 suggests that the conversion to 20:4n-6 may be limited following burn injury.

N-3 fatty acids are known to have significant effects on immune functions involving inflammation (Blok et al.,1996; Cerra,1991). Each PL fraction exhibited alterations in n-3 content during the post burn period. Changes in n-3 content of neutrophils have been reported to alter functional parameters ((Lee et al.,1985) (Gyllenhammar et al.,1986)as they do in other immune cells (Robinson et al., 1998; Wu et al.,1996). It is unknown whether these patients would utilize additional n-3 fatty acids or if there are impairments in n-3 fatty acid metabolism that would preclude supplementation in the diet.

Membrane fluidity (Tomita-Yamaguchi et al.,1991; Traill et al.,1986), adherence, binding of opsonins, phagocytosis and release of granules by neutrophils are related to membrane composition (Arturson, 1985(Bates et al.,1995; Deitch,1984). Changes in the proportions of SFA, MUFA and PUFA observed in this study would be expected to alter physical properties of the membrane and could impact on cell to cell communication, adherence, migration, phagocytosis and other membrane associated functions of neutrophils, such as NADPH oxidase assimilation, as they do with other immune cells (Calder et al., 1991; Tomita-Yamaguchi et al., 1991; Traill et al., 1986). Changes in number and affinity of receptor binding sites important in stimulating oxidative burst are related to the fatty acid composition of the membrane. Increased linoleic acid in cells, such as that observed in the PS fraction neutrophils in this study, has been reported to increase oxidative burst in rats (Gyllenhammar et al.,1990). These studies demonstrate a relationship between dietary fat and neutrophil function that are possibly mediated through membrane compositional changes.

Neutrophils isolated between 12 and 19 days post burn injury (t2) in this study had a two fold higher oxidative burst ratio after stimulation than any other timepoint. Our results suggest that 2 weeks post-injury, neutrophils may be primed in response to circulating agents such as cytokines and lipid derived factors (Condliffe et al., 1998). The oxidative burst of neutrophils has been reported to be both decreased (el-Falaky et al.,1985; Gadd et al., 1989; Rosenthal et al.,1996) and increased (Bjerknes et al.,1989; de Chalain et al.,1994; Shih et al.,1999) after burn injury. Differences in these studies could be due to methodologies used to isolate neutrophils, stimulate the oxidative burst, time of post burn sampling or patient groups utilized. It is known that isolation techniques can alter the physical properties of neutrophils (Sengelov et al.,1994), therefore this study employed methods that avoid excessive handling procedures.

In addition to an increased oxidative burst, neutrophils had significantly fewer granules at t2 compared to other timepoints and also demonstrated significant reductions in granule content after 10 minutes of stimulation with PMA suggesting an inability to synthesize new granules after they have been released as has been described after burn (Griswold,1993). Decreased concentrations of granular enzymes have been reported in bacterial infection, surgical trauma (Fletcher et al.,1990) and during the second week post burn (Arturson,1985) corresponding to t2 in this study and results in abnormal chemotaxis and bactericidal killing (Alexander et al., 1978). Little is known about how granularity may be affected by lipid composition, however, membrane fusion of secretory vesicles as well as granule content (Deitch,1984) and release are dependent on membranes (Sengelov et al., 1994; Tapper,1996). Increased degranulation has been reported with physiological levels of 20:4n-6 *in vitro* (Bates et al., 1995). Neutrophils released into circulation prematurely from the bone marrow, reported to occur post burn (Wakefield et al.,1993), have both increased oxidative burst and reduced enzyme content (Simms et al.,1989), characteristics of neutrophils isolated between 12 and 19 days (t2) in this study. Granularity and production of superoxide reflect intracellular killing capacity of neutrophils and abnormalities in these effector functions may contribute to post burn infections and other complications reported to occur most commonly at 2 weeks post burn (Gadd et al., 1989).

In conclusion, neutrophils demonstrated increased oxidative burst activity after stimulation between 12 and 19 days post burn (Gadd et al., 1989). It is likely neutrophils were primed *in vivo* for this increased response *in vitro*. The decreased levels of 20:4n-6 in membranes, also observed <19 days post injury, increased with recovery. Other phospholipid compositional changes such as increased n-6 and n-3 fatty acids and reduced n-6/n-3 ratios could also influence the oxidative burst and granularity following burn. Our data suggests that this decrease may be due to factors other than the availability of dietary precursors. The possibility of reduced conversion of 18:2n-6 to 20:4n-6 and 20:4n-6 release early after burn injury for synthesis of lipid derived inflammatory mediators cannot be ruled out. Reducing the n-6 or increasing n-3 content of the diet may reduce 20:4n-6 availability, however, alterations in the activity of delta-6-desaturase, PLA_2 or other lipid pathways after burn injury may override possible diet effects. Further work is need to develop appropriate nutritional intervention strategies designed to modulate fatty acid composition of neutrophils to reduce the harmful effects of the oxidative burst while maintaining important infection defense mechanisms following burn injury.

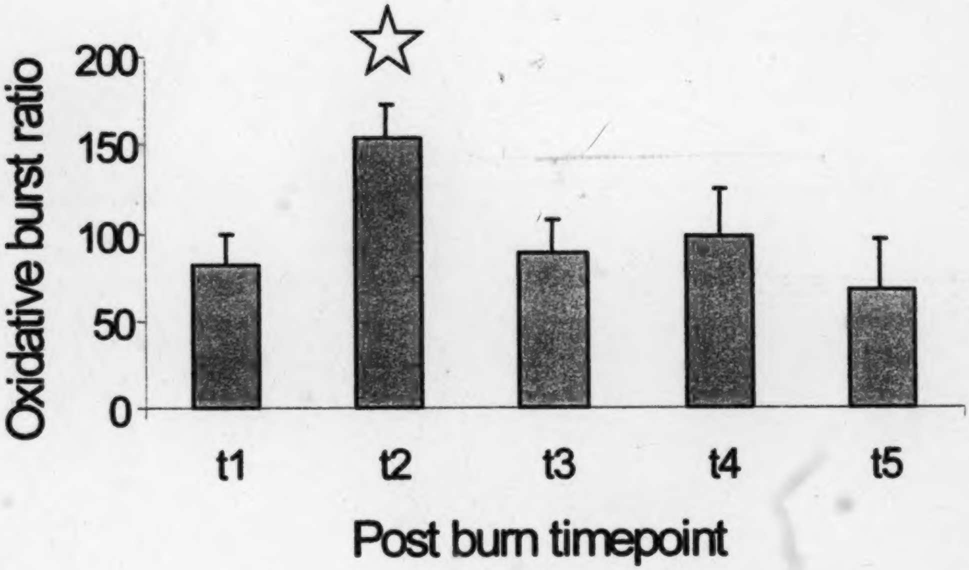


FIGURE III.1: Effect of time post burn injury on oxidative burst ratio of neutrophils. Mean channel fluorescence of gated cells was determined using flow cytometry to measure reduction of the DHR substrate by oxidative compounds released by the activated neutrophils after 10 minutes of stimulation with PMA *in vitro*. Oxidative burst ratio is calculated as: mean channel fluorescence at 10 minutes / mean channel fluorescence at 0 minutes. Bars represent mean ± SEM ($n \leq 10$). Significant differences ($p<0.05$) from the first timepoint (t1) following burn injury were determined using a repeated measures ANOVA and are indicated in the figure with a star.

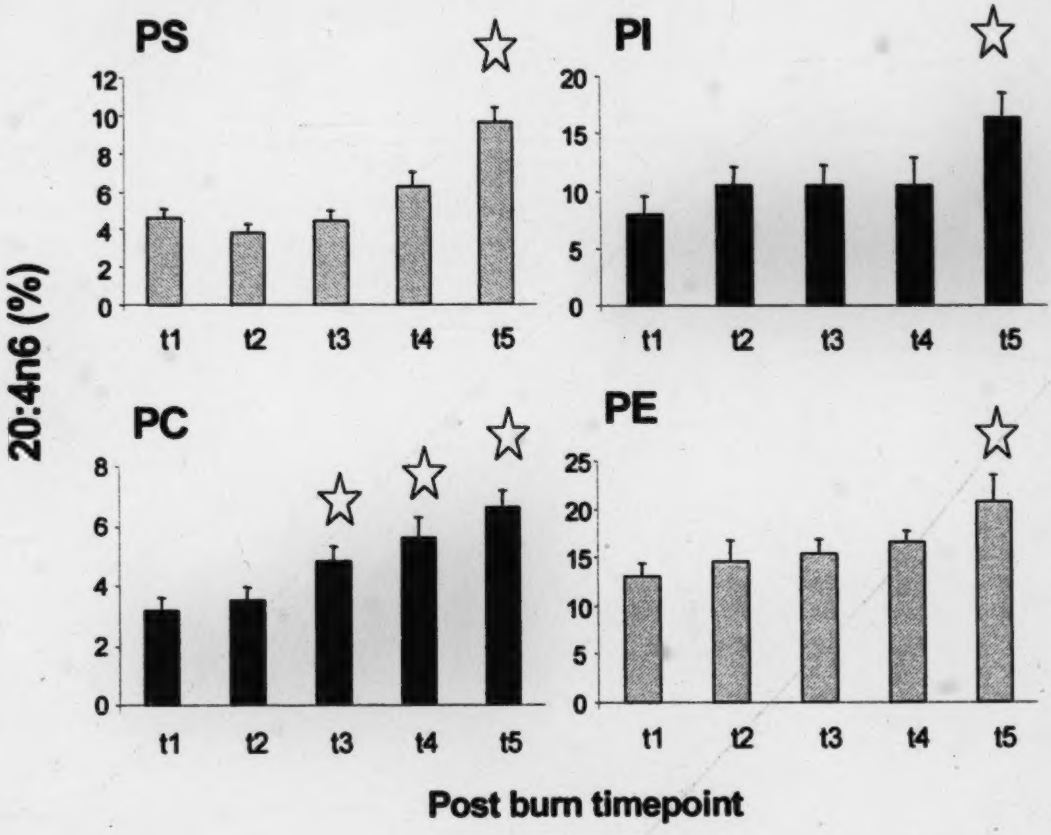


FIGURE III.2: Effect of time post burn injury on total 20:4n6 content (% of total fatty acids) of the major PL fractions of neutrophils. Bars represent mean ± SEM (n≤10). Significant differences ($p<0.05$) from the first timepoint (t1) following burn injury were determined using a repeated measures ANOVA and are indicated in the figure with a star.

Table III.1

Subject ID	*Sex*	*Age (Years)*	*TBSA Burn (%)*	*Burn Type*	*Length of hospital stay*	*Daily Energy Intake (kcal)*	*Daily fat intake (kcal)*	*Daily n6 intake (g)*	*Daily n3 intake (g)*	*Daily carbohydrate intake (g)*	*Daily protein intake (g)*
01	F	23	90%	Flame	59 days	2411	699.2	23	0.6	311	117
02	M	20	45%	Flame	82 days	2550	739.5	25	0.7	329	123
03	M	25	55%	Flame	22 days	2247	651.6	22	0.6	290	116
04	M	63	35%	Hot tar	36 days	2130	617.7	21	0.5	275	103
05	M	30	22%	Flame	19 days	1913	554.8	19	0.5	253	95
06	M	65	20%	Flame	24 days	2125	616.3	21	0.5	281	105
07	F	46	15%	Flame	16 days	1722	499.4	17	0.4	228	85
08	M	51	18%	Electric	13 days	2025	587.3	20	0.5	268	100
09	M	57	60%	Flame	98 days	2792	809.7	27	0.7	345	133
10	M	24	12%	Flame	14 days	1913	554.8	19	0.5	253	95
Mean		*40 ± 6 years*	*37 ± 8%*		*38 ± 10 days*	*2183 ± 103 kcal*	*633 ± 30 kcal*	*21 ± 1 g*	*0.6 ± 0.02 g*	*283 ± 12 g*	*107 ± 5 g*

TABLE III.1: Characteristics of patients included in the study and their mean daily dietary intake. Enteral feeds were stopped when feeding complications persisted and 6 hours prior to surgery, therefore values include those periods of reduced intake and estimate average daily intake when Nitro-Pro™ provided the majority of the energy consumed. TBSA, total body surface area.

Table III.2A

Nutrition information	*Per 1000 ml*
Calories	1240
Osmolality	310 mOsm/kg water
Protein (% of total calories)	60 g (19%)
Protein source	Caseinates*
Fat (% of calories)	40 g (29%)
Fat source	50% corn oil, 50% MCT
Carbohydrates (% of total calories)	160 g (52%)
Carbohydrate source	maltodextrin
Fiber	N/A
Free Water	770 ml
Nonprotein:Calorie Ratio	104:1
Calorie:nitrogen ratio	129:1

*Complete protein source

TABLE III.2A: Nutritional composition of Nitro-Pro™. Source: *Nutrition Medical, Inc. Minneapolis, MN*

Table III.2B

Vitamins and Minerals	*Per 1000 ml*
Vitamin A	4000 I.U
Vitamin C	144 mg
Thiamine	1.2 mg
Riboflavin	1.4 mg
Niacin	16 mg
Calcium	800 mg
Iron	14.4 mg
Vitamin D	320 I.U
Vitamin E	24 I.U
Vitamin B_6	1.6 mg
Phosphorus	0.3 mg
Iodine	120 µg
Magnesium	320 mg
Zinc	12 mg
Copper	1.6 mg
Biotin	0.2 mg
Pantothenic Acid	8 mg
Mangenese	2 mg
Choline	200 mg
Sodium	30 mEq
Potassium	30 mEq
Chloride	31 mEq
Vitamin K	80 µg
Selenium	120 µg
Chromium	120 µg
Molybdenum	240 µg

TABLE III.2B: Vitamin and mineral composition of Nitro-Pro™. Nitro-Pro™ contains 80% of the USDA recommendations for water soluble vitamins and minerals and 240% requirement of Vitamin C. Source: *Nutrition Medical, Inc. Minneapolis, MN.*

Table III.3

Fatty acid	*g/1000 ml*
C 8:0	14.62
C 10:0	5.76
C 12:0	0.21
C 14:0	0.02
C 16:0	2.29
C 17:0	0.02
C 18:0	0.42
C 18:1	4.64
C 18:2	11.58
C 18:3	0.32
C 20:0	0.07
C 22:0	0.02

Fat per serving: 10g/250 ml

TABLE III.3: Fatty acid composition of Nitro-Pro™. The fat content was composed of half medium chain triglycerides and the other half corn oil for a total of 29% of total energy coming from fat. Source: *Nutrition Medical, Inc. Minneapolis, MN.*

Table III.4A

Nutrient Analysis	*Per 5 g serving (1 Scoop)*
Energy	28 kcal
Protein (Source)	5 g (whey concentrate, soy lecithin)
Fat	≤ 0.60g
Carbohydrates	≤ 0.67g
Water	≤ 0.60g
Calcium	23 mg
Sodium	13 mg
Potassium	65 mg
Phosphorus	≤ 22 mg

Table III.4B

Amino Acid	*mg/5g protein*
Essential	
Histidine	83
Isoleucine	292
Leucine	542
Lysine	458
Methionine	125
Phenylalanine	167
Threonine	375
Tryptophan	83
Valine	292
Nonessential	
Alanine	250
Arginine	125
Aspartic Acid	542
Cystine	125
Glutamic Acid	917
Glycine	125
Proline	333
Serine	292
Tyrosine	167

TABLE III.4: Composition of Pro-Mod™ protein supplement

Source: *Ross Products Division, Saint-Laurent, PQ*

Table III.5

Postburn time	*0 minutes*	*5 minutes*	*10 minutes*	*15 minutes*
		(Mean FSC)		
t1	626 ± 26^{a}	645 ± 28^{a}	646 ± 30^{a}	657 ± 29^{a}
t2	573 ± 26^{ac}	599 ± 28^{ab}	590 ± 30^{ab}	579 ± 29^{ac}
t3	466 ± 26^{b}	529 ± 28^{b}	533 ± 30^{b}	486 ± 29^{b}
t4	518 ± 39^{bc}	591 ± 42^{ab}	603 ± 46^{ab}	564 ± 43^{ab}
t5	467 ± 35^{b}	505 ± 37^{b}	499 ± 41^{b}	482 ± 38^{bc}

TABLE III.5 : Effect of burn injury and recovery on size of neutrophils before and after stimulation with PMA. Size of neutrophils was determined by measuring mean forward scatter (FSC) of gated cells using flow cytometry before and after stimulation with PMA for 5, 10 and 15 minutes in vitro. Data are expressed as means ± SEM ($n \leq 10$). Significant differences during recovery were determined using a repeated measures ANOVA and values within a column not sharing common superscripts are significantly ($p<0.05$) different.

Table III.6

Postburn time	*0 minutes*	*5 minutes*	*Index*	*10 minutes (Mean SSC)*	*Index*	*15 minutes*	*Index*
t1	680.5 ± 24.3^{ab}	659.6 ± 24.8^{ac}	0.98 ± 0.02^{ab}	614.5 ± 25.5^{a}	0.91 ± 0.02^{a}	615.0 ± 28.4^{a}	0.91 ± 0.02^{a}
t2	628.6 ± 24.3^{a}	613.2 ± 24.8^{a}	0.96 ± 0.02^{a}	578.5 ± 25.5^{a}	$0.93.\pm 0.02^{ac}$	620.4 ± 28.4^{a}	0.93 ± 0.02^{ab}
t3	734.0 ± 24.3^{b}	770.7 ± 24.8^{b}	1.03 ± 0.02^{b}	745.2 ± 25.5^{b}	0.96 ± 0.02^{abc}	736.8 ± 28.4^{b}	0.97 ± 0.02^{b}
t4	669.1 ± 36.9^{ab}	619.3 ± 37.6^{a}	1.02 ± 0.03^{ab}	635.1 ± 38.7^{ac}	0.98 ± 0.03^{bc}	600.8 ± 43.0^{a}	0.96 ± 0.03^{ab}
t5	742.3 ± 32.6^{b}	723.6 ± 33.2^{bc}	0.98 ± 0.03^{ab}	708.2 ± 34.2^{bc}	1.01 ± 0.026^{b}	691.4 ± 38.1^{ab}	1.03 ± 0.02^{c}

TABLE III.6: Effect of burn injury and recovery on granularity of neutrophils before and after stimulation with PMA. Neutrophil granularity was determined by measuring mean side scatter (SSC) of gated cells using flow cytometry. Data are expressed as means ± SEM ($n \leq 10$). Significant differences during recovery were determined using a repeated measures ANOVA and values not sharing common superscripts in the same column are significantly ($p<0.05$) different.

Table III.7

Post burn Time	*PC*	*PE*	*PS*	*PI*
	(% of total)			
t1	3.2 ± 0.4^{a}	12.9 ± 1.6^{a}	4.6 ± 0.5^{ab}	8.0 ± 1.5^{a}
t2	3.5 ± 0.4^{a}	13.3 ± 1.7^{ab}	3.8 ± 0.5^{a}	10.5 ± 1.6^{a}
t3	4.8 ± 0.5^{b}	14.5 ± 1.9^{ab}	4.4 ± 0.6^{ab}	10.5 ± 1.7^{a}
t4	5.6 ± 0.7^{bc}	13.6 ± 2.6^{ab}	6.2 ± 0.8^{b}	10.5 ± 2.4^{ab}
t5	6.6 ± 0.6^{c}	19.0 ± 2.3^{b}	9.6 ± 0.8^{c}	16.4 ± 2.1^{b}
Reference (n=6)	*7.8 ±0.8*	*19.1 ±5.0*	*5.4 ±2.2*	*12.4 ±3.6*

TABLE III.7: Effect of burn injury and recovery on 20:4n6 content of major neutrophil phospholipid fractions. Total 20:4n6 content in PL fractions of neutrophils isolated at specific times during recovery from burn injury was determined using TLC and GC analysis and is expressed a percent of total fatty acids. Data are expressed as means ± SEM (n ≤10). Differences during recovery were determined using a repeated measures ANOVA and values not sharing common superscripts are significantly ($p<0.05$) different.

Table III.8

Post burn time	*PC*	*PE*	*PS*	*PI*
	(18:2n-6 / 20:4n-6 Ratio)			
t1	4.2 ± 0.6^{a}	1.3 ± 0.4	1.5 ± 0.3^{a}	0.9 ± 0.2
t2	4.5 ± 0.6^{a}	0.8 ± 0.4	1.3 ± 0.3^{a}	0.4 ± 0.2
t3	2.5 ± 0.8^{ab}	0.5 ± 0.5	1.0 ± 0.3^{a}	0.6 ± 0.2
t4	3.0 ± 1.1^{ab}	0.5 ± 0.6	0.8 ± 0.4^{b}	0.5 ± 0.3
t5	1.2 ± 0.9^{b}	0.3 ± 0.6	0.1 ± 0.4^{b}	0.4 ± 0.2

TABLE III.8: The 18:2n-6/20:4n-6 ratio in phospholipid fractions (separated by thin layer chromatography) of neutrophils isolated at specific times during recovery from burn injury was determined by gas chromatography analysis. Data are expressed as means ± SEM ($n \leq 10$). Significant differences during recovery were determined using a repeated measures ANOVA and values within a column not sharing common superscripts are significantly ($p<0.05$) different.

Table III.9

Fatty Acid	t1	t2	t3	t4	t5
			(% fatty acids)		
18:2(6)	9.1 ± 1.3	10.7 ± 1.7	9.4 ± 2.0	12.0 ± 2.9	9.5 ± 1.8
18:3(6)	0.3 ± 0.2	0.1 ± 0.1	0.1 ± 0.0	0.3 ± 0.1	nil*
20:2(6)	0.7 ± 0.3	0.9 ± 0.3	0.8 ± 0.2	0.9 ± 0.2	0.6 ± 0.2
20:3(6)	0.7 ± 0.1^{a}	1.0 ± 0.3^{ab}	1.1 ± 0.2^{ab}	1.7 ± 0.3^{b}	1.5 ± 0.1^{b}
20:4(6)	3.2 ± 0.4^{a}	3.5 ± 0.4^{a}	4.8 ± 0.5^{b}	5.6 ± 0.7^{bc}	6.6 ± 0.6^{c}
22:4(6)	0.3 ± 0.1^{a}	0.4 ± 0.2^{ab}	0.4 ± 0.2^{ab}	1.0 ± 0.1^{b}	0.6 ± 0.2^{ab}
22:5(6)	0.1 ± 0.0	nil*	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.0
Σn6	14 ± 1	17 ± 1	17 ± 1	14 ± 2	14 ± 2
18:3(3)	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	0.6 ± 0.3
18:4(3)	0.1 ± 0.0	0.1 ± 0.0	0.4 ± 0.2	0.1 ± 0.0	0.1 ± 0.0
20:5(3)	0.7 ± 0.2	0.8 ± 0.3	0.8 ± 0.6	0.5 ± 0.1	1.0 ± 0.6
22:5(3)	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.7 ± 0.2	0.4 ± 0.1
22:6(3)	0.4 ± 0.1	0.4 ± 0.1	0.7 ± 0.3	0.8 ± 0.2	0.6 ± 0.1
Σn3	2 ± 0.3^{a}	2 ± 0.3^{a}	3 ± 0.4^{ab}	3 ± 0.4^{ab}	3 ± 0.4^{b}
ΣPUFA	17 ± 1^{a}	18 ± 1^{a}	19 ± 1^{a}	23 ± 1^{b}	22 ± 1^{b}
ΣSFA	48 ± 1	48 ± 1	49 ± 2	47 ± 2	48 ± 2
ΣMUFA	35 ± 1^{a}	25 ± 1^{a}	34 ± 1^{a}	30 ± 1^{b}	28 ± 1^{b}

*nil=<0.04%

TABLE III.9: Fatty acid composition of the PC fraction of neutrophils isolated at specific times during recovery from burn injury was determined by gas chromatography analysis. Data are expressed as means ± SEM (n ≤10). Significant differences during recovery were determined using a repeated measures ANOVA and values within a row not sharing common superscripts are significantly ($p<0.05$) different.

Table III.10

Fatty Acid	t1	t2	t3 *(% fatty acids)*	t4	t5
18:2(6)	5.3 ± 0.5	5.0 ± 1.1	5.1 ± 0.7	5.8 ± 1.0	4.2 ± 0.7
18:3(6)	0.2 ± 0.1	0.3 ± 0.3	0.2 ± 0.1	0.7 ± 0.7	0.2 ± 0.1
20:2(6)	0.8 ± 0.4	0.4 ± 0.2	0.5 ± 0.3	0.9 ± 0.3	0.8 ± 0.2
20:3(6)	1.7 ± 0.3	3.0 ± 1.0	3.0 ± 0.6	3.6 ± 0.8	3.0 ± 0.7
20:4(6)	8.0 ± 1.2^{a}	10.7 ± 1.7^{ab}	11.1 ± 1.4^{ab}	11.6 ± 2.0^{ab}	17.5 ± 3.9^{b}
22:3(6)	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	1.2 ± 1.2	nil*
22:4(6)	0.4 ± 0.2	0.6 ± 0.3	0.8 ± 0.2	0.9 ± 0.3	0.9 ± 0.3
22:5(6)	0.6 ± 0.2	0.4 ± 0.3	0.7 ± 0.4	0.1 ± 0.1	0.3 ± 0.3
Σn6	17 ± 2^{a}	21 ± 2^{ab}	19 ± 2^{ab}	21 ± 3^{ab}	25 ± 3^{b}
18:3(3)	0.3 ± 0.2	0.4 ± 0.3	0.5 ± 0.4	0.4 ± 0.2	0.4 ± 0.3
18:4(3)	nil^{a}	0.1 ± 0.1^{ab}	0.2 ± 0.1^{ab}	0.1 ± 0.1^{ab}	0.3 ± 0.2^{b}
20:5(3)	2.7 ± 0.9	1.8 ± 0.8	1.1 ± 0.5	1.3 ± 1.1	0.7 ± 0.6
22:5(3)	0.2 ± 0.1	0.5 ± 0.3	0.2 ± 0.1	0.2 ± 0.1	0.4 ± 0.2
22:6(3)	0.2 ± 0.1^{a}	0.2 ± 0.1^{a}	0.5 ± 0.2^{ab}	1.0 ± 0.5^{b}	0.8 ± 0.3^{ab}
Σn3	3 ± 1	3 ± 1	3 ± 1	5 ± 1	4 ± 1
ΣPUFA	22 ± 2	24 ± 2	22 ± 3	26 ± 4	29 ± 3
ΣSFA	61 ± 1^{a}	56 ± 2^{b}	62 ± 2^{a}	51 ± 3^{b}	59 ± 2^{ac}
ΣMUFA	17 ± 1	18 ± 1	17 ± 1	19 ± 2	16 ± 2

*nil=<0.04%

TABLE III.10: Fatty acid composition of the PI fraction of neutrophils isolated at specific times during recovery from burn injury was determined by gas chromatography analysis. Data are expressed as means ± SEM (n ≤10). Significant differences during recovery were determined using a repeated measures ANOVA and values within a row not sharing common superscripts are significantly ($p<0.05$) different.

Table III.11

Fatty Acid	t1	t2	t3	t4	t5
			(% fatty acids)		
18:2(6)	4.0 ± 0.5^{a}	3.9 ± 0.5^{ab}	4.2 ± 0.6^{a}	4.2 ± 0.7^{a}	2.5 ± 0.3^{b}
18:3(6)	0.2 ± 0.2^{a}	1.0 ± 0.7^{b}	0.1 ± 0.1^{ab}	0.4 ± 0.3^{ab}	0.1 ± 0.1^{ab}
20:2(6)	0.4 ± 0.2	1.4 ± 1.3	0.3 ± 0.2	1.5 ± 1.1	2.4 ± 2.1
20:3(6)	1.5 ± 0.4	0.8 ± 0.3	1.5 ± 0.3	1.8 ± 0.2	1.9 ± 0.4
20:4(6)	4.6 ± 0.5^{ab}	3.8 ± 0.5^{a}	4.4 ± 0.6^{ab}	6.2 ± 0.8^{b}	9.6 ± 0.8^{c}
22:3(6)	0.1 ± 0.1	0.2 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.2
22:4(6)	0.8 ± 0.5	0.3 ± 0.2	0.6 ± 0.2	0.9 ± 0.2	1.4 ± 0.3
22:5(6)	0.3 ± 0.1	0.5 ± 0.5	0.7 ± 0.5	nil*	0.3 ± 0.1
Σn6	12 ± 1^{a}	11 ± 1^{a}	12 ± 1^{a}	14 ± 1^{ab}	17 ± 1^{b}
18:3(3)	0.3 ± 0.2^{ab}	0.6 ± 0.3^{a}	0.1 ± 0.1^{b}	0.2 ± 0.2^{ab}	0.6 ± 0.5^{ab}
18:4(3)	0.2 ± 0.1^{a}	0.9 ± 0.6^{b}	nil^{a*}	nil^{a*}	0.1 ± 0.1^{a}
20:5(3)	1.4 ± 0.4	2.4± 1.2	1.2 ± 0.6	0.4 ± 0.3	0.2 ± 0.1
22:5(3)	0.2 ± 0.1	0.9 ± 0.4	0.8 ± 0.4	0.5 ± 0.2	1.4 ± 0.7
22:6(3)	0.5 ± 0.3	0.2 ± 0.1	0.4 ± 0.3	0.6 ± 0.1	0.9 ± 0.3
22:6(3)	0.5 ± 0.3	0.2 ± 0.1	0.4 ± 0.3	0.6 ± 0.1	0.9 ± 0.3
Σn3	2 ± 1	5 ± 1	3 ± 1	3 ± 2	4 ± 2
ΣPUFA	15 ± 1^{a}	17 ± 1^{ab}	14 ± 2^{a}	17 ± 2^{ab}	21 ± 2^{b}
ΣSFA	33 ± 2	32 ± 2	32 ± 2	29 ± 3	32 ± 3
ΣMUFA	51 ± 2	51 ± 2	52 ± 2	51 ± 3	48 ± 3

*nil=<0.04%

TABLE III.11: Fatty acid composition of the PS fraction of neutrophils isolated at specific times during recovery from burn injury was determined by gas chromatography analysis. Data are expressed as means ± SEM (n ≤10). Significant differences during recovery were determined using a repeated measures ANOVA and values within a row not sharing common superscripts are significantly ($p<0.05$) different.

Table III.12

Fatty Acid	t1	t2	t3 *(% fatty acids)*	t4	t5
18:2(6)	8.8 ± 1.7	9.3 ± 0.6	8.3 ± 1.0	10.2 ± 1.1	6.9 ± 0.6
18:3(6)	0.1 ± 0.1	0.3 ± 0.2	0.1 ± 0.1	0.2 ± 0.2	0.3 ± 0.2
20:2(6)	0.5 ± 0.1	0.7 ± 0.3	0.3 ± 0.2	0.8 ± 0.1	0.8 ± 0.4
20:3(6)	1.2 ± 0.2	2.2 ± 0.3	2.2 ± 0.5	2.3 ± 0.3	1.7 ± 0.4
20:4(6)	13.6 ± 1.4^{a}	14.5 ± 2.2^{ab}	15.4 ± 1.7^{ab}	16.6 ± 1.8^{ab}	20.6 ± 3.9^{ab}
22:3(6)	0.1 ± 0.1	0.1 ± 0.1	nil*	0.1 ± 0.1	0.1 ± 0.1
22:4(6)	2.2 ± 1.0	2.7 ± 1.3	2.4 ± 0.9	4.2 ± 0.4	5.7 ± 1.6
22:5(6)	0.3 ± 0.2	0.4 ± 0.1	0.5 ± 0.2	0.5 ± 0.1	0.8 ± 0.4
Σn6	26 ± 2	27 ± 2	27 ± 2	29 ± 3	33 ± 3
18:3(3)	0.4 ± 0.1^{a}	1.3 ± 0.3^{b}	1.0 ± 0.4^{b}	1.1 ± 0.2^{ab}	1.0 ± 0.3^{ab}
18:4(3)	0.5 ± 0.2	0.2 ± 0.1	0.5 ± 0.2	0.5 ± 0.1	0.4 ± 0.2
20:5(3)	1.8 ± 0.7	1.2 ± 0.4	1.0 ± 0.4	0.5 ± 0.2	0.8 ± 0.4
22:5(3)	1.9 ± 0.4	1.7 ± 0.7	1.4 ± 0.5	2.2 ± 0.4	2.0 ± 0.4
22:6(3)	1.4 ± 0.4	1.3 ± 0.7	1.9 ± 0.7	2.0 ± 0.5	2.0 ± 0.6
Σn3	6 ± 1	7 ± 1	6 ± 1	6 ± 1	6 ± 1
ΣPUFA	30 ± 27^{a}	32 ± 2^{ab}	37 ± 2^{b}	37 ± 3^{bc}	44 ± 2^{c}
ΣSFA	37 ± 2	35 ± 2	39 ± 2	38 ± 3	31 ± 2
ΣMUFA	29 ± 1^{a}	29 ± 1^{a}	26 ± 1^{b}	26 ± 1^{b}	25 ± 1^{b}

*nil=<0.04%

TABLE III.12: Fatty acid composition of the PE fraction of neutrophils isolated at specific times during recovery from burn injury was determined by gas chromatography analysis. Data are expressed as means ± SEM (n ≤10). Significant differences during recovery were determined using a repeated measures ANOVA and values within a row not sharing common superscripts are significantly ($p<0.05$) different.

E. References

Alexander JW, Ogle CK, Stinnett JD et al. A sequential, prospective analysis of immunologic abnormalities and infection following severe thermal injury. Ann Surg 188: 809-816, 1978.

Alexander JW, Stinnett JD, Ogle CK et al. A comparison of immunologic profiles and their influence on bacteremia in surgical patients with a high risk of infection. Surg 86: 94-104, 1979.

Arturson G. Neutrophil granulocyte functions in severely burned patients. Burns Incl Therm Inj 11: 309-319, 1985.

Bates, E. J., Ferrante A, Smithers L, Poulos A, and Robinson BS. Effect of fatty acid structure on neutrophil adhesion, degranulation and damage to endothelial cells. Atherosclerosis 116:247-259, 1995

Bialick R, Gill R, Berke G et al. Modulation of cell-mediated cytotoxicity function after alteration of fatty acid composition in vitro. J Immunol 132: 81-87, 1984.

Bjerknes R, Vindenes H, Pitkanen J et al. Altered polymorphonuclear neutrophilic granulocyte functions in patients with large burns. J Trauma-Inj Infect Crit Care 29: 847-855, 1989.

Bjornson AB, Somers SD, Knippenberg RW et al. Circulating factors contribute to elevation of intracellular cyclic- 3',5'-adenosine monophosphate and depression of superoxide anion production in polymorphonuclear leukocytes following thermal injury. J Leukoc Biol 52: 407-414, 1992.

Blok WL, Katan MB, van der Meer JW. Modulation of inflammation and cytokine production by dietary (n- 3) fatty acids. J Nutr 126: 1515-1533, 1996.

Boyum A. Isolation of mononuclear cells and granulocytes from human blood. Scand.J.Clin.Lab.Invest. 21 (suppl):77-89, 1968

Braquet M, Lavaud P, Dormont D et al. Leukocytic functions in burn-injured patients. Prostaglandins 29: 747-764, 1985.

Calder PC, Bond JA, Bevan SJ et al. Effect of fatty acids on the proliferation of concanavalin A- stimulated rat lymph node lymphocytes. Int J Biochem 23: 579-588, 1991.

Cerra FB. Nutrient modulation of inflammatory and immune function. Am J Surg 161: 230-234, 1991.

Chapkin RS and Carmichael SL. Effects of dietary n-3 and n-6 polyunsaturated fatty acids on macrophage phospholipid classes and subclasses. Lipids 25: 827-834, 1990.

Condliffe AM, Kitchen E, Chilvers ER. Neutrophil Priming: pathophysiological consequences and underlying mechanisms. Clin Sci 94: 461-471, 1998.

Davis JM, Dineen P, Gallin JI. Neutrophil degranulation and abnormal chemotaxis after thermal injury. J Immunol 124: 1467-1471, 1980.

de Chalain TM, Bracher M, Linley W et al. Cytoskeletal actin: the influence of major burns on neutrophil structure and function. Burns 20: 416-421, 1994.

Deitch EA. The relationship between thermal injury and neutrophil membrane functions as measured by chemotaxis, adherence and spreading. Burns Incl Therm Inj 10: 264-270, 1984.

Diboune M, Ferard G, Ingenbleek Y et al. Composition of phospholipid fatty acids in red blood cell membranes of patients in intensive care units: effects of different intakes of soybean oil, medium-chain triglycerides, and black-currant seed oil. JPEN J Parenter Enteral Nutr 16: 136-141, 1992.

Dobke MK, Deitch EA, Harnar TJ et al. Oxidative activity of polymorphonuclear leukocytes after thermal injury. Arch Surg 124: 856-859, 1989.

Duque RE, Phan SH, Hudson JL et al. Functional defects in phagocytic cells following thermal injury. Application of flow cytometric analysis. Am J Pathol 118: 116-127, 1985.

el-Falaky MH, Abdel-Hafez A, Houtah AH. Phagocytic activity of polymorphonuclear leucocytes in burns. Burns Incl Therm Inj 11: 185-191, 1985.

Field CJ. Modulation of adipocyte membrane composition and insulin action in normal and diabetic states by dietary fat. 1988. University of Alberta.

Field CJ, Toyomizu M, Clandinin MT. Relationship between dietary fat, adipocyte membrane composition and insulin binding in the rat. J Nutr 119: 1483-1489, 1989.

Fletcher J, Haynes AP, Crouch SM. Acquired abnormalities of polymorphonuclear neutrophil function. Blood Rev 4: 103-110, 1990.

Folch J, Lees M, and Stanley SGH. A simple method for the isolation and purification of total lipids from animal tissues. J.Biol.Chem. 226:497-509, 1957

Fujishima S and Aikawa N. Neutrophil-mediated tissue injury and its modulation. Int Care Med 21: 277-285, 1995.

Gadd MA and Hansbrough JF. The effect of thermal injury on murine neutrophil oxidative metabolism. J Burn Care Rehab 10: 125-130, 1989.

Gerster H. The use of n-3 PUFAs (fish oil) in enteral nutrition. Int J Vitam Nutr Res 65: 3-20, 1995.

Gottschlich MM and Alexander JW. Fat kinetics and recommended dietary intake in burns. JPEN J Parenter Enteral Nutr 11: 80-85, 1987.

Griswold JA. White blood cell response to burn injury. Semin Nephrol 13: 409-415, 1993.

Grogan JB and Miller RC. Impaired function of polymorphonuclear leukocytes in patients with burns and other trauma. Surg Gynecol Obstet 137: 784-788, 1973.

Gyllenhammar H, Hafstrom I, Borgeat P et al. Dietary linoleate supplementation modulates formyl-peptide receptor expression and functional responses of rat neutrophils. J Lab Clin Med 115: 487-496, 1990.

Gyllenhammar H, Ringertz B, Becker W et al. Effects of essential fatty acid deficiency on rat neutrophils. Immunol.Lett. 13:185-9, 1986

Hansbrough JF, Wikstrom T, Braide M et al. Neutrophil activation and tissue neutrophil sequestration in a rat model of thermal injury. J Surg Res 61: 17-22, 1996.

Heck EL, Edgar MA, Hunt JL et al. A comparison of leukocyte function and burn mortality. J Trauma 20: 75-77, 1980.

James MJ, Gibson RA, D'Angelo M et al. Simple relationships exist between dietary linoleate and the n-6 fatty acids of human neutrophils and plasma. Am J Clin Nutr 58: 497-500, 1993.

Jordan JE, Zhao Z-Q, Vinten-Johansen J. The role of neutrophils in myocardial ischemia - reperfusion injury. Cardiovasc Res 43: 860-878, 1999.

Leape L. Initial changes in burns; tissue changes in burned and unburned skins of rhesus monkeys. J Trauma 10: 488-492, 1970.

Lee TH, Hoover RL, Williams JD et al. Effect of dietary enrichment with eicosapentaenoic and docosahexaenoic acids on in vitro neutrophil and monocyte leukotriene generation and neutrophil function. N Engl J Med 312: 1217-1224, 1985.

Mahadevappa VG and Powell WS. The metabolism of arachidonic and eicosapentaenoic acids in human neutrophils stimulated by A23187 and FMLP. J Cell Biochem 40: 341-352, 1989.

Matzner Y. Acquired neutrophil dysfunction and diseases with an inflammatory component. Sem Hematol 34: 291-302, 1997.

Palombo JD, Demichele SJ, Lydon E et al. Cyclic vs continuous enteral feeding with omega-3 and gamma- linolenic fatty acids: effects on modulation of phospholipid fatty acids in rat lung and liver immune cells. JPEN J Parenter Enteral Nutr 21: 123-132, 1997.

Peck MD. Omega-3 polyunsaturated fatty acids: benefit or harm during sepsis? New Horiz 2: 230-236, 1994.

Robinson LE and Field CJ. Dietary long-chain (n-3) fatty acids facilitate immune cell activation in sedentary, but not exercise-trained rats. J Nutr 128: 498-504, 1998.

Rosenthal J, Thurman GW, Cusack N et al. Neutrophils from patients after burn injury express a deficiency of the oxidase components p47-phox and p67-phox. Blood 88: 4321-4329, 1996.

Rosenthal MD, Gordon MN, Buescher ES et al. Human neutrophils store type II 14-kDa phospholipase A2 in granules and secrete active enzyme in response to soluble stimuli. Biochem Biophys Res Commun 208: 650-656, 1995b.

Rosenthal MD, Rzigalinski BA, Blackmore PF et al. Cellular regulation of arachidonate mobilization and metabolism. Prost Leukot Essent Fatty Acids 52: 93-98, 1995a.

Saffle JR, Wiebke G, Jennings K et al. Randomized trial of immune-enhancing enteral nutrition in burn patients. J Trauma 42: 793-800, 1997.

Sayeed MM. Neutrophil signaling alteration: an adverse inflammatory response after burn shock. Medicina 58: 386-392, 1998.

Sengelov H, Boulay F, Kjeldsen L et al. Subcellular localization and translocation of the receptor for N- formylmethionyl-leucyl-phenylalanine in human neutrophils. Biochem J 299: 473-479, 1994.

Shih HC, Su CH, Lee CH. Superoxide production of neutrophils after severe injury: impact of subsequent surgery and sepsis. Am J Emerg Med 17: 15-18, 1999.

Simms HH. Polymorphonuclear leukocytes: their role as central cellular elements in shock pathogenesis. Shock 4:225-231, 1995

Simms HH, Frank MM, and Quinn TC. Studies on Phagocytosis in patients with acute bacterial infections. J.Clin.Invest. 83:252-260, 1989

Smolen JE. Characteristics and mechanisms of secretion by neutrophils In: The Neutrophil: Cellular Biochemistry and Physiology, Hallett, M B pp. 23-61.CRC press, Boca Raton, Florida ,1989.

Tapper H. The secretion of preformed granules by macrophages and neutrophils. J Leukoc Biol 59: 613-622, 1996.

Tomita-Yamaguchi M, Rubio C, Santoro TJ. Regional influences on the physical properties of T cell membranes. Life Sci 48: 433-438, 1991.

Touchstone JC, Chen JC, Beaver KM. Improved separation of phospholipids in thin layer chromatography. Lipids 15: 61, 1980.

Traill KN and Wick G. Lipids and Lymphocyte Function. Immunol Today 5: 20-76, 1986.

Vowells SJ, Sekhsaria S, Malech HL et al. Flow cytometric analysis of the granulocyte respiratory burst: a comparison study of fluorescent probes. J Immunol Methods 178: 89-97, 1995.

Wakefield CH, Carey PD, Foulds S et al. Polymorphonuclear leukocyte activation. An early marker of the postsurgical sepsis response. Arch Surg 128: 390-395, 1993.

Warden, GD. Fluid Resuscitation In: Fluid Resuscitation and Early Management in Total Burn Care, Herndon, D H pp. 53-60,1973.

Wu D, Meydani SN, Meydani M et al. Immunologic effects of marine- and plant-derived n-3 polyunsaturated fatty acids in nonhuman primates. Am J Clin Nutr 63: 273-280, 1996.

IV.

Alterations in Lymphocyte Function and Relation to Phospholipid Composition Following Burn Injury

A. Introduction

Impaired immune functions involved in both acquired and innate immunity can predispose patients to infection, a major complication following burn injury (Alexander,1986; Sayeed,1996). Post burn immunosuppression is associated with a reduced ability of lymphocytes to proliferate in response to mitogens, impaired interleukin-2 (IL-2) production and receptor expression, dysregulated cytokine production and changes in immune cell phenotypes (Barlow,1994; O'Riordain et al.,1995; O'Sullivan et al.,1997; Teodorczyk-Injeyan et al.,1986). Natural killer cells and macrophages produce and respond to IFN-γ (Markovic et al.,1993; Tripp et al.,1993), an important pro-inflammatory cytokine. IL-2 and IFN-γ are cytokines produced by the T-helper 1 (TH1) and T-cytotoxic 1 (TC1) subsets of lymphocytes (Mosmann et al.,1996). Burn injury has been reported to inhibit TH1 responses and promote a TH2 response thereby reducing effector responses against bacterial infections (Lyons et al.,1997; O'Sullivan et al.,1995).

Lipids and their products have been shown to affect macrophage activation, NK cell cytotoxicity (de Pablo et al.,1998; Yaqoob et al.,1994), cytokine production (de Pablo et al.,2000) and proliferation of lymphocytes both *in vitro* and *in vivo* (Calder,1999). Fatty acid composition of membrane phospholipids (PL) has been reported to influence the physical properties of immune cell membranes and impact on cell growth, maturation, transformation, transport, enzyme activity, expression of cell surface antigens and cytotoxic potential of T cells (Tomita-Yamaguchi et al.,1991; Traill et al.,1986). Additionally, PL have been shown to be involved in lymphocyte activation signals (Traill et al., 1986), and serve as precursors for prostaglandins that exert suppressive effects on proliferation and cytokine production (Kinsella et al.,1990). Despite the effects of lipids on immune function having been extensively studied, the changes in PL fatty acid composition and their relationship to lymphocyte function following burn injury have not been investigated. Many studies have examined nutritional intervention

---

A version of this chapter has been submitted for publication. Pratt et al., 2001. Critical Care Medicine.

strategies aimed at improving outcome measures in critically ill patients (Bower et al.,1995; Cerra et al.,1991; Daly et al.,1992; Gottschlich et al.,1990; Kemen et al.,1995; Saffle et al.,1997; Weimann et al.,1998), however, the beneficial effects of specific fatty acids are difficult to determine when other immunomodulating nutrients are included in these formulations (Section I.F.5). Before the optimal fat content in clinical diets aimed at improving immune function can be recommended for burn patients, an understanding of how burn injury affects fatty acid composition of immune cell membranes and how this might impact on immune function is required. The current study was designed to characterize lymphocyte related immune functions as well as alterations in the lymphocyte phospholipids immediately following and during recovery from burn injury to characterize the changes in fatty acid composition most consistent with immunological recovery.

B. Methods

1. Materials

Sodium ^{51}chromate and [^{3}H]-thymidine were obtained from Amersham Canada (Oakville, ON, Canada). RPMI 1640 culture media (with glutamine) was purchased from Fisher Scientific (Edmonton, AB, Canada). Fetal calf serum (FCS) and antibiotic-antimytotic were purchased from Gibco (Burlington, ON, Canada). All other culture ingredients, Concanavalin A (Con A), Phorbol Myristate Acetate (PMA), Phytohematogluttinin (PHA) and Ecolite® scintillation fluid were obtained from ICN (Montreal, PQ, Canada). The K562 cell line was purchased from American Type Culture Collection (Rockville, MD). H-plates were purchased from Analtech (Newark, DE) and solvents were purchased from VWR (Edmonton, AB, Canada). Bovine serum albumin (BSA, Fraction V), trypan blue, ficoll hypaque gradients, chemicals for buffers and all other lipid supplies, including standards, were purchased from Sigma Chemicals (St. Louis, MO).

2. Subjects

Ethical approval, subjects and sample collection were described in Section III.B.2.

3. Preparation of Lymphocytes

Warmed sterile lysis buffer was added to whole blood to deplete red blood cells. After gently inverting for 5 minutes, cells were pelleted and washed twice with sterile KRH+0.5% BSA. Cells were prepared and cultured in complete culture media (CCM; RPMI supplemented with 50 g/L FCS; 2.5 µmol/L 2-mercaptoethanol, 100 units/ml penicillin, 100 µg/mL streptomycin, and 25 mmol/L HEPES). Lymphocytes to be used for lipid analysis and NK cell cytotoxicity were isolated using ficoll hypaque gradient centrifugation as previously described in Section III.B.6. After the lymphocyte gradient was removed, cells were washed three times with buffer described above and prepared in RPMI supplemented as described above. Cell viability for both isolation methods was assessed using trypan blue exclusion and was greater than 99% for all samples. Cells to be used for the NK cytotoxic assay were removed from the suspension and set aside. The remaining cells were pelleted and frozen immediately at –70°C until phospholipid analysis was performed.

4. Mitogenic Responses of Immune Cells

Under sterile conditions, mixed lymphocyte cultures (1.25 x 10^9/L) in the media described above plus 2-mercaptoethanol (2.5 µmol/L) were cultured in 96 well microtiter plates in triplicate without mitogen or with either Con A (5 mg/L), PMA (40µg/L) or PHA (5 ηg/L). Plates were covered and incubated in humidified 5% CO_2 atmosphere at 37°C for 42, 66 and 90 hours. Eighteen hours before harvesting the cells, each well was pulsed with of $[^3H]$ thymidine (18.5 kBq/well). Cells were harvested on glass fibre filters using a multiwell harvester (Skatron, Lier, Norway), transferred to scintillation vials containing Ecolite® and counted using a Beckman beta counter (LS 5801®, Beckman Instruments Inc., Missisauga, ON, Canada). Thymidine incorporation into cultured cells was estimated using the mean total dpm for the triplicate wells and stimulation indices (SI) were calculated using the formula:

$$SI = \frac{\begin{array}{c}([^3H]\text{ thymidine (dpms) incorporated by stimulated cells})\\ -\,([^3H]\text{ thymidine (dpms) incorporated by unstimulated cells})\end{array}}{([^3H]\text{ thymidine incorporated by unstimulated cells})}$$

When the number of cells obtained from patients were insufficient to perform the proliferation assay with all mitogens at each incubation timepoint, PHA and the 90 h timepoint were excluded.

5. Mononuclear Cell Phenotyping

A non-sterile 96 well v-bottom microtiter plate was conditioned with phosphate buffered saline containing FCS (40 g/L; wash buffer) for at least 20 minutes prior to starting the assay. Whole blood (100 µl) was added each well and red blood cells were lysed by adding 100 µl warm lysis buffer to each well, pipetting gently to mix and leaving at room temperature for 3 minutes. Plates were centrifuged for 2 minutes at 1500 rpm, supernatants removed and lysing procedure repeated until red blood cells were not visible in the pellet. Plates were washed once with wash buffer (200 µl). Immune cell subsets were characterized by immunofluorescence assay using supernatants from hybridomas secreting mouse monoclonal antibodies (mAb) specific for the different human immune cell subsets. Table IV.1 describes the specificity of each monoclonal antibody used. All mAb were purchased pre-labelled in phosphate buffered saline (pH 7.4) containing bovine serum albumin and 0.1% (w/v) sodium azide. MAb concentrations were optimized prior to use and aliquoted accordingly. Just prior to use, antibodies were prepared in PBS and 10 µl of each antibody was added to the designated well. Table I.V.2 describes the three-colour combinations that were used to identify the lymphocyte subsets. Antibodies were added to the wells, plates were sealed, covered with foil and incubated for 30 minutes at 4°C. Plates were washed once in 200 µL of wash buffer and incubated for another 30 minutes at 4°C with the streptavidin conjugate (10 µl) in wells contained biotinylated mAb. Cells were washed twice and fixed in phosphate buffered saline containing paraformaldehyde (10 g/L plus Na azide) and analyzed on a FACScan® (Becton-Dickinson, Sunnyvale, CA) according to the relative fluorescence intensity using CellQuest software. Resulting percentages were corrected for background fluorescence (0-5%) determined by incubating the cells with appropriate isotype controls (IgG1, IgG2a, IgG2b).

6. NK Cell Cytotoxicity

NK cell cytotoxicity was determined on isolated lymphocytes from each patient using a 4 h chromium release assay described by Field *et al.* (Field et al.,1991). NK sensitive K562 cells (6 x 10^6) were incubated with sodium chromate (^{51}Cr, 5.55 MBq) in 100% FCS (total volume=1 ml) in a shaking water bath (37°C) for 1 hour. Cells were washed with cold, sterile PBS and pelleted by centrifuging for 10 minutes at 1000 rpm. The wash was repeated twice more, supernatant removed and cells resuspended in 5 ml complete culture media (CCM, described above) with 20% (v/v) FCS and counted for viability using trypan blue exclusion. Cells were then diluted to a final density of 0.1 x 10^6 cells/ml in CCM. Cells (50 µl) were seeded into a 96 well v-bottom microtiter plate. Lymphocytes, isolated using ficoll-hypaque gradients as described above, were added in triplicate to the wells containing labelled K562 cells at effector:target ratios of 2:1, 10:1, 25:1, 50:1 and 100:1 when cell numbers were sufficient. When the number of cells obtained from a patient were insufficient to perform the planned effector:target ratios, only the 100:1 ratio was performed in triplicate. Spontaneous release was determined from target cells incubated in the absence of effector cells and maximum release was determined from detergent lysis of labelled target cells using CCM with 10% (v/v) FCS and 4% (v/v) Triton-X (BDH chemicals, Toronto, ON). Plates were covered and centrifuged up to 1000 rpm. Plates were incubated for 4 h in 5% CO_2 atmosphere at 37°C. Plates were spun at 1000 rpm for 10 minutes and an aliquot (75 µl) of the supernatant from each well was transferred into borosilicate glass tubes and counted in a Gamma counter (Beckman Gamma 8000®, Beckman Instruments, Inc. Mississauga, ON, Canada) to determine the extent of target cell lysis. Cytotoxicity was calculated as follows:

$$\text{\% Specific Lysis} = \quad 100 \times \left[\frac{\text{(experimental release-spontaneous release)}}{\text{(maximum release-spontaneous release).}} \right]$$

7. Cytokine Production

Under sterile conditions, mixed lymphocyte cultures in the media described above were prepared at a concentration of $1\text{x}10^6$ cells/ml in 4 ml sterile plastic tubes. Con A (5 mg/L) and PHA (5 ηg/L) were added to tubes at the same concentration used for the proliferation assay. Unstimulated cytokine production was estimated by adding the same amount of RPMI supplemented with fetal calf serum (50 g/L). Tubes were covered and incubated in humidified 5% CO_2 atmosphere at 37°C for 48 h. Cells were pelleted by centrifuging tubes for 5 minutes at 1500 rpm. Supernatants from each tube were transferred to sterile microcentrifuge tubes using plastic transfer pipets and frozen immediately at –70°C. Supernatants were later thawed and concentrations of IL-2 and IFN-γ were determined using commercially purchased ELISA kits (Pharmingen, Mississauga, ON).

8. Cytokine Determination

Purified anti-cytokine capture antibody was diluted 1:250 in coating buffer and 100 μL added to each well of Nunc Maxisorp P/N microwell plate. Plates were sealed, incubated overnight at room temperature and washed 3 times with 200 μL wash buffer. After the last wash, plates were inverted and blotted on an absorbent surface. Non-specific binding was blocked by adding 200 μl of assay diluent, sealing the plate and incubating at room temperature for 1 h. Plates were then washed 3 times with wash buffer as previously described. Standards (7.8 ρg/ml to 500 ρg/ml) were prepared in assay diluent and 100 μL of standard and 100 μL of sample were added to each well in duplicate. Plates were sealed and incubated for 2 h at room temperature. Plates were washed five times as described above. Biotinylated anti-cytokine detecting solution was prepared in assay diluent and 100 μL was added to each well. Plates were sealed and incubated at room temperature for 1 h. Plates were washed 7 times with wash buffer and 100 μl of substrate solution (tetramethylbenzidine) were added to each well. Plates were left unsealed at room temperature for 30 minutes in the dark. Stop solution (50 μl) was added to each well and the optical density was read at 450 ηm on a plate reader (Model EL309; Bio-Tek Instruments Inc., Burlington, VT).

9. Fatty Acid analysis

A modified Folch method was used to extract lipids from isolated lymphocytes as previously described for neutrophils in Section III.B.7. Individual PL were separated on thin layer chromatography and standards were used to identify the major PL fractions (PC, PE, PS and PI). Bands were scraped and methylated as described in detail in Section III.B.7. Fatty acid methyl esters were separated by automated gas liquid chromatography as previously described in Section III.B.7. Total PUFA, SFA and MUFA as well as percent of 20:4n-6, n-6 and n-3 fatty acids were determined. The 18:2n-6/20:4n-6 ratio was calculated to estimate delta-6 and delta-5-desaturase and elongase activities, in addition to the n-6/n-3 fatty acid ratio.

10. Statistical Analysis

Data is reported as mean ± SEM. To determine differences between PL composition and immune parameters at post burn timepoints burn injury, a repeated measures analysis of variance was used using the post burn time periods described above. Subjects were grouped into groups based on size of burn (large= >35%, n=5 and small=<35%, n=5) and when differences existed between these two groups, the results were presented. When there were no differences between groups, all subjects were included in the analysis and overall mean presented. Significant differences ($p<0.05$) between time periods were identified using least square means. The number of subjects samples at each timepoint was as follows t1, n=10; t2, n=10; t3, n=8; t4, n=6; t5, n=8. Subjects with burns >35% TBSA were sampled at every timepoint. To determine if subjects followed the same pattern of changes in lymphocyte function and phospholipid composition during recovery, orthoganol comparisons of the linear ($y=mx+b$; time after burn = x) and quadratic equations were used. To determine associations between cytokine production, immune phenotypes, proliferation and lipid composition, specific correlations were tested. All statistical analyses were conducted using the SAS statistical package (Version 6.12, SAS Institute, Cary, NC).

C. Results

1. Dietary Intake

Dietary intake of the subjects was described in Section III.C.1 and shown in Table III.1.

2. Phenotypes in peripheral blood

There was a significant increase in the proportion of CD8+ cells in peripheral blood from t1 to t5 but no significant change in relative proportion of CD4 cells (Table IV.3) nor the CD4/CD8 ratio. There were no differences in the expression of CD45RA or CD45RO on either T cell subset during recovery, however, the ratio of CD45RO to CD45RA cells on CD4 cells decreased at t2 (Table IV.4). A higher percentage of CD8 cells expressed CD71 and CD25 at t2 than at t3 (Table IV.5). There was significantly ($p<0.05$) higher expression of CD25 on the CD45RO subset at t2 (45 ± 5%) compared to t1 (31 ± 4%; data not shown). There were no changes in expression of activation markers on the CD45RA subset throughout recovery.

The relative percent of NK cells, determined by the presence of CD11c on the cell surface decreased from early (t1 and t2) to late (t5) post burn timepoints (Table IV.3). Proportion of cells that were CD14+CD11c+ (macrophages/monocytes) did not change during burn recovery (data not illustrated). However, the proportion of activated macrophages (CD16+CD14+CD11c+) was higher at t2 compared to t5 (Table IV.3) The proportion of B cells (CD20+) was higher at early (t1) compared to late (t4 and t5) post burn timepoints (Table IV.3).

3. Estimated proliferation

There were no differences between post burn time points in the rate of [^{3}H] thymidine incorporation after 42 h or 90 hours of stimulation with Con A (42 hours=29882 ± 10926 dpms; 90 hours= 38697 ± 6622 dpms) or PMA (42 hours=15289 ± 9097dpms; 90 hours=19412 ± 2893). Maximal [^{3}H] thymidine incorporation occurred at 66 hours for the mitogens tested at all post burn time points. At 66 hours, unstimulated [^{3}H] thymidine incorporation was significantly higher at early (t2) compared to late post burn timepoints (Table IV.6). The stimulation index measuring proliferative responses to

Con A and PMA was significantly greater at t3 versus t2 and t1 respectively. Production of IL-2 and IFN-γ by stimulated cells was positively correlated with proliferative responses to both Con A and PMA at 66 hours (Table IV.6). There were no significant differences in proliferative responses or stimulation indexes at 42 h (24383 ± 11253; SI= 13.82 ± 6.7) or 66 h (48347.6 ± 4040.3; SI= 52.77 ± 8.3) with PHA.

4. Natural Killer Cell Cytotoxicity

Subjects with smaller burn injury (less than 20% TBSA burns) were not included in the NK analysis as it was not possible to perform the NK measurement at every timepoint post burn. NK cell activity at the 100:1 ratio was lowest at t1 compared to all the other timepoints (Figure IV.1). Percent specific lysis at the 100:1 ratio was significantly increased at t2 compared to t1 (Figure IV.1), even though numbers of NK cells did not change (Table IV.3).

5. Cytokine Production

IFN-γ and IL-2 production in unstimulated cultures were undetectable. Production of these two cytokines in response to Con A (IFN-γ: 117.3 ± 43.2 ρg/ml and IL-2: 75.7 ± 42.6 ρg/ml) and PHA (IFN-γ 181.4 ± 50.1 ρg/ml and IL-2: 74.7 ± 37.1 ρg/ml) did not differ significantly between post burn timepoints. IFN-γ production in response to PHA correlated negatively with percent n-3 fatty acids in the PS fraction ($r=0.7$, $p=0.008$). IFN-γ production in response to PHA and Con A was positively correlated with percent 20:4n-6 in the PC ($r=0.6$, $p=0.04$) and PE fraction ($r=0.06$, $p=0.05$) of lymphocyte membrane lipids, respectively.

6. Phospholipid analysis

The most consistent change observed in lymphocyte PL was the significantly lower content of 20:4n-6 in all phospholipid fractions early after burn (Figure IV.2). Compared to t1, the 20:4n-6 content increased at t5 in the PC fraction, at t3 in the PS fraction and at t2 in the PE and PI fractions. Total n-6 fatty acids increased significantly from t1 to t5 in the PS and PE fractions (Table IV.9, Table IV.10). In the PC, PS and PI fractions, the n-3 fatty acid content decreased significantly at t2 (Tables IV.7-9). The

18:3n-3 and 20:5n-3 content of PC were highest at t1 (Table IV.7) and significantly greater than at t2 and t3. Both 18:3n-3 and 20:5n-3 content of PE were higher at t5 than at t1 (Table IV.10) but there were no other changes in individual n-3 fatty acids in any PL fraction. The n-6/n-3 ratio increased from t1 to t5 in all fractions (Figure IV.3). PC was the only fraction that increased in SFA content during recovery whereas the PS (Table IV.8), PE (Table IV.10) and PI (Table IV.9) fractions decreased in SFA content from early to late burn recovery timepoints. MUFA content was lower at t5 than at t2 in the PC (Table IV.7) and PE (Table IV.10) fractions. The 18:2/20:4n-6 ratio in the PC and PS fractions was lower at t5 compared to t1 (Table IV.11). Complete fatty acid profiles of lymphocyte PL fractions following burn injury and those of healthy individuals are given in Appendices IV.AP1-AP5.

D. Discussion

This study provides evidence for immunosuppression early after burn followed by activation of immune function corresponding to the second to third week post injury. This study also demonstrates a relationship between the PL composition of lymphocytes and their functions. Higher expression of CD25 on CD8+ and CD45RO+ cells, and CD71 on CD8+ cells, increased NK cytotoxicity, higher percent of activated macrophages, and a higher unstimulated proliferative response provide evidence for immunostimulation at t2. At the same time (t2), content of 20:4n-6 increased and n-3 fatty acid content decreased in major PL fractions.

Lipids are known to impact on lymphocyte functions both directly through changes in physical properties of the membrane and indirectly through their role as precursors for second messengers (de Pablo et al., 2000; O'Sullivan et al., 1997; Sayeed,1996). The n-6/n-3 ratio, an indicator of membrane fluidity (Tebbey et al.,1992; Traill et al., 1986) has been reported to alter functional parameters in lymphocytes (Jeffery et al.,1996; Kelley et al.,1993; Wu et al.,1996). All PL fractions of lymphocytes in this study exhibited an increase in the n-6/n-3 ratio from early to late post burn. This suggests changes in the availability or use of the n-6 or n-3 fatty acids in elongase and desaturase pathways (Cetinkale et al.,1997). In the present study, lymphocyte membrane phospholipids exhibited a low content of 20:4n-6 early after burn that increased with recovery. PI and PE in lymphocyte membranes contain the largest proportions of 20:4n-6, however, the values observed at t1 were half that observed in healthy individuals (PI=28.0 ± 2.3% and PE=22.1 ± 4.6%; Appendix IV.AP5). These two fractions had significant increases in 20:4n-6 content after only 12 days. The depletion of 20:4n-6 observed in all fractions in the immediate post burn period suggests that a proportion of 20:4n-6 was used for synthesis of inflammatory mediators derived from 20:4n-6 (Gerster,1995). Higher 18:2n-6/20:4n-6 ratios early after burn could also suggest slow conversion to 20:4n-6 from 18:2n-6. Consistent with this, inhibition of delta-6-desaturase activity has been reported post burn (Braquet et al.,1985). The supplemental diet fed provided low levels of n-3 fatty acids, therefore, competitive inhibition by n-3 lipids is not a likely reason for lower 20:4n-6 levels. Consumption of diets rich in linoleic acid, such as the clinical diet fed to the subjects in this study, would be expected to increase PUFA, particularly 20:4n-6 in cell membranes (Lokesh et al.,1992). However, the n-6 fatty acid content in lymphocyte PL was lower early after burn injury when energy intake from the enteral diet was the highest. This suggests that the reduced PUFA, particularly 20:4n-6, early after burn injury is likely the result of the injury on metabolism or absorption rather than a dietary deficiency. In support of this, alterations in intestinal lipases (Carter et al.,1994), lipoprotein metabolism and carrier proteins necessary for lipid transport (Coombes et al.,1980; Vega et al.,1988) have been reported to occur post burn (Birke et al.,1965; Coombes et al., 1980; Gottschlich et al.,1987).

The role of dietary n-3 fatty acids in reducing inflammatory responses is well established (Blok et al.,1996; Cerra,1991). IFN-γ is a key cytokine in inflammatory responses and is produced by the TH1 and TC1 subsets of lymphocytes (Mosmann et al., 1996). This study found a strong negative correlation between the n-3 fatty acid content in the PS fraction of lymphocyte membranes and IFN-γ production by stimulated lymphocytes. At t2, the n-3 fatty acid content of PS decreased while 20:4n-6 content of PE increased, both of which were significantly associated with increased IFN-γ production.

It is well established that IL-2 production is decreased following burn injury whereas the effect on receptor expression is more controversial (O'Riordain et al., 1995) with both no change (O'Riordain et al., 1995) increases (Schluter et al.,1991a) and decreases (Faist et al.,1993; Gadd et al.,1989; Wood et al.,1984) being reported. The present study examined IL-2 receptor expression on unstimulated cells and IL-2 production by stimulated cells. There was increased expression of the IL-2 receptor on CD8 and the RO+ subset in unstimulated cells and [^{3}H]-thymidine incorporation was the highest (t2), suggesting *in vivo* activation (Schluter et al.,1991b). Studies by others (Teodorczyk-Injeyan et al.,1991) have suggested a strong *in vivo* activation of lymphocytes post burn. *In vivo* T cell activation has been reported to be associated with reduced T cell proliferation *in vitro* (Teodorczyk-Injeyan et al., 1991). This is consistent with the response to Con A in the present study at t2. CD8+ lymphocytes, like CD4 cells, have been shown to produce different cytokine profiles (Mosmann et al., 1996). This study suggests it is the CD8 cells that may mediate burn injury immunosuppression as suggested by their increased expression of antigen priming and activation molecules (CD25, CD71). CD45RO+ cells are capable of proliferating in response to IL-2 and providing help for activation of immune responses (Akbar et al.,1991). Consistent with this, the highest expression of CD45RO on lymphocytes was observed when proliferative responses increased. Higher CD45RA expression has been associated with hypo-responsiveness of lymphocytes (Lightstone et al.,1993) and is consistent with the observation of a reduced proliferative response at t2. The CD4$^+$CD45RA$^+$ subset of lymphocytes has been reported to suppress immune responses by inducing CD8 suppressor activity (Clement,1992). Reduced CD45RO/CD45RA ratio on CD4+ cells in this study corresponded with increased expression of activation markers on CD8 cells and reduced [^{3}H]-thymidine incorporation in response to Con A.

Proliferation responses were positively correlated with production of IFN-γ and IL-2 in this study. IFN-γ and IL-2 are cytokines produced by the CD4+ TH1 subset of cells (O'Sullivan et al., 1995) and stimulate delayed type hypersensitivity responses and inflammation (O'Sullivan et al., 1995). There have been reports of TH2 cell type being predominant after burn injury (Lyons et al., 1997; O'Sullivan et al., 1995), which would reduce T cell proliferation *in vitro*. There is recent evidence that dietary polyunsaturated fats decrease TH1 but not TH2 responses (Wallace et al.,1999). Involvement of a TH2 response may be supported by an increased percentage of B cells early after burn.

Burn injury has been reported to depress NK cell cytotoxicity (Blazar et al.,1986) which can last up to 50 days in severely injured patients. NK cells have also been reported to be affected by changes in fatty acids and the metabolites derived from them (Chang et al.,1989; Oxholm et al.,1992; Seaman et al.,1984). NK cytotoxicity in this study was reduced when the 18:2n-6/20:4n-6 ratio of lymphocytes was the highest. Increased 18:2n-6 content of lymphocytes has been shown to increase the sensitivity of NK cell function to the inhibitory action of prostaglandins (Oxholm et al., 1992). The early depression of NK cytotoxicity may be attributed to the stress response (Blazar et al., 1986) or surgical procedures (Pollack et al.,1991). NK cells defend against transformed self cells and viruses and can also directly lyse infected cells (Bancroft,1993), therefore, reduced cytotoxicity may place the patient at risk of viral infection. IL-2 and IFN-γ increase the cytotoxic capabilities of NK cells (Singh et al.,1992). Inhibition of TH1 responses reported in the early post burn period (O'Sullivan et al., 1995) would be expected to reduce NK cytotoxicity. NK cells can stimulate the production of IFN-γ by T-lymphocytes (Chan et al.,1992). Improved NK cytotoxicity was observed at the same time (t2) as higher proliferative responses to mitogens that were positively associated with IL-2 and IFN-γ production, and changes in the content of 20:4n-6 and n-3 content that were associated with increased IFN-γ production, were observed.

Higher percent of activated monocytes observed early after burn in this study supports other investigators who have reported increased macrophage production of inflammatory mediators following burn injury (Fukushima et al.,1994; Grbic et al.,1991; Ogle et al.,1990; Waymack et al.,1989). Fatty acids released from lymphocyte membranes via activation of phospholipase can be used by macrophages (Schultz,1991) to produce prostaglandins (Fukushima et al., 1994; Ogle et al.,1993). Macrophages are activated by a TH1 response and also stimulate the production of IFN-γ and IL-2 by lymphocytes and NK cells (Romagnani,1995).

This study demonstrates significant alterations in phenotypes, innate immune cell activation and proliferative responses following burn injury. The innate immune system appears to be activated at 12-19 days post injury as evidenced by improved NK cytotoxicity and higher percent of activated monocytes. These responses appear to precede activation of acquired immune defenses. Although the production of TH1 cytokines was not different between post burn timepoints, several other indices would indicate that stimulation of a TH1 response begins to occur at t2 as well. Proliferative responses, which increased at 12-19 days and 21-35 days, were associated with IL-2 and IFN-γ, cytokines produced by TH1 subset of lymphocytes. IL-2 and IFN-γ have been reported to activate NK cells which also occurred between 12 and 19 days post injury in this study. The immunosuppression observed early after burn appears to be mediated by the CD8 subset of cells which have also been shown to produce cytokine profiles similar to that of TH1 cells. There were no differences in proliferative responses or phenotypes from the third week to later post burn timepoints. Significant changes in fatty acid composition of lymphocyte PL, specifically the 20:4n-6 and n-3 content, also occurred between 12 and 19 days and may relate to functional changes observed at this timepoint. This study provides evidence of alterations in lymphocyte PUFA metabolism in the early post burn period with decreases in 20:4n-6 in lymphocyte membranes as a result of release or impaired synthesis. These results also suggest that the regulation in synthesis, incorporation and release of fatty acids from each fraction differs, exemplifying the importance of measuring fatty acid composition in isolated PL fractions. Further work is needed to dissect the complex relationships between immune responses and fatty acid metabolism in immune cells following burn injury.

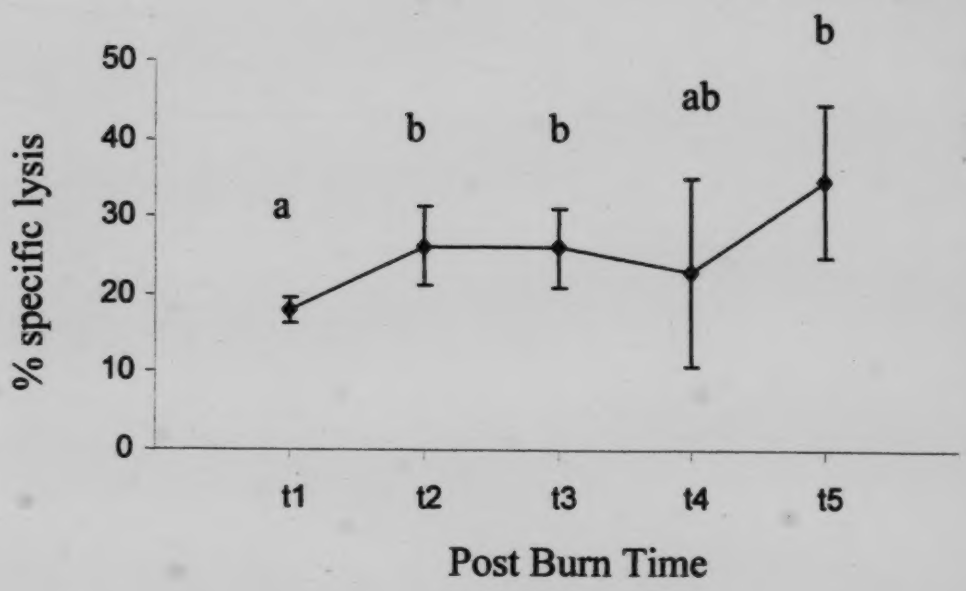


FIGURE IV.1: Natural killer cell cytotoxicity at the 100:1 effector/target ratio at post burn timepoints. Data are expressed as means ± SEM (n=5). NK cell cytotoxicity was determined by incubating isolated mononuclear cells from each patient with NK sensitive K562 cells labelled with sodium chromate and determining the extent of target cell lysis. Spontaneous release was determined from target cells incubated in the absence of effector cells. Cytotoxicity was calculated as follows: % Specific lysis = 100 x (experimental release-spontaneous release) / (maximum release-spontaneous release). Significant differences between post burn timepoints were determined using a repeated measures ANOVA and data points not sharing common superscripts are significantly ($p<0.05$) different.

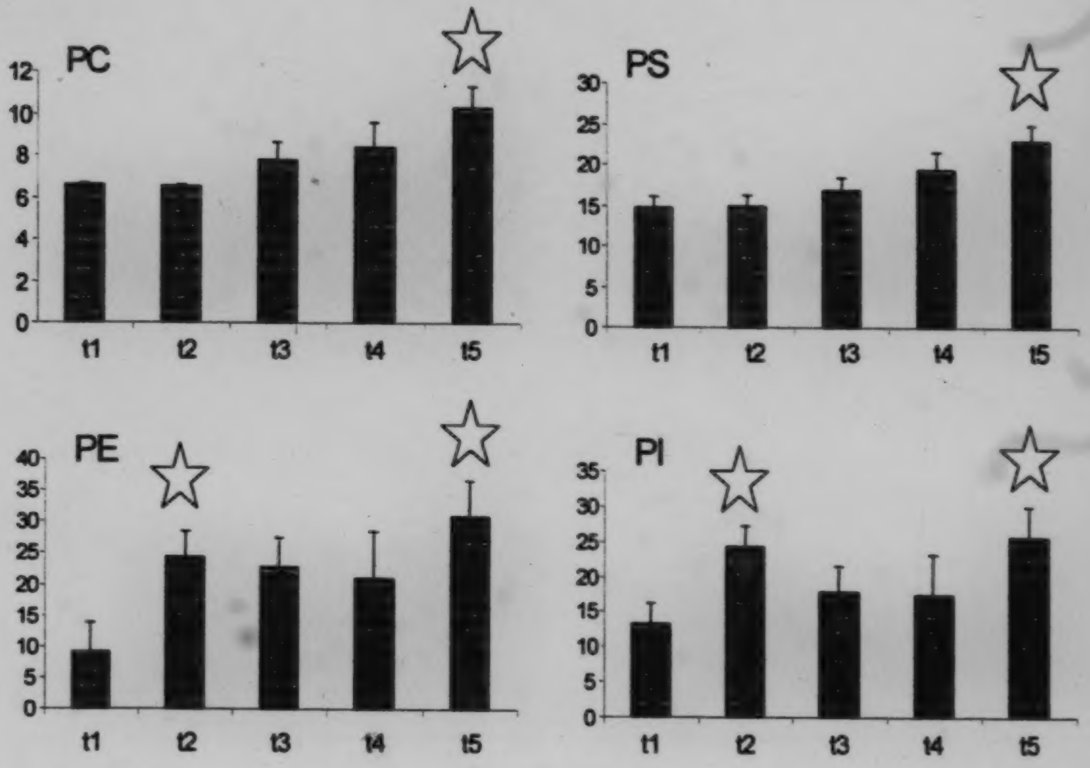


FIGURE IV.2: 20:4n-6 content in major PL fractions of lymphocytes at post burn timepoints. Bars represent mean ± SEM (n ≤10). Significant differences between post burn timepoints were determined using a repeated measures ANOVA and significant differences ($p<0.05$) from the first timepoint post burn (t1, <12 days) are indicated in the figure with a star.

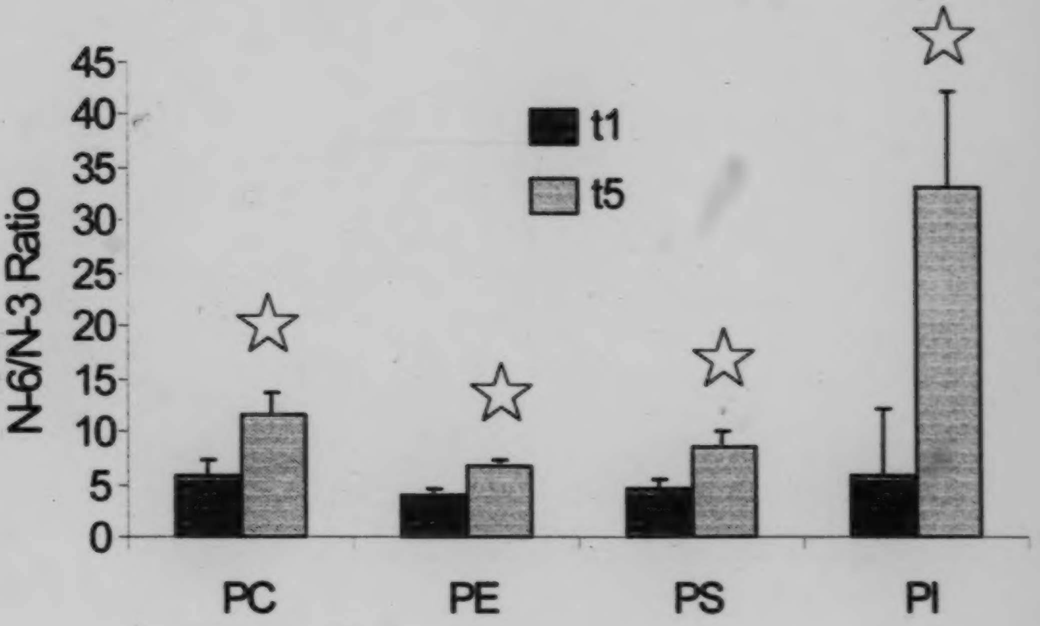


FIGURE IV.3: The n-6/n-3 ratio in major PL fractions of lymphocytes at the first (t1) and final (t5) post burn timepoints. Bars represent mean ± SEM (n ≤10). Significant differences between post burn timepoints were determined using a repeated measures ANOVA and significant differences ($p<0.05$) from the first timepoint post burn (t1, <12 days) are indicated in the figure with a star.

Table IV.1

Monoclonal Antibody (Label)	*Isotype*	*Specific for*	*Company Purchased from*
CD4 (FITC, B)	IgG1	helper/inducer T lymphocytes	Sigma Chemicals[a]
CD8 (r-PE, B)	IgG2a	cytotoxic/suppressor T lymphocytes	Sigma Chemicals[a]
CD3 (B)	IgG2b	TCR receptor, T cells	Serotec[b]
CD45RO (r-PE, FITC)	IgG2a	LCA for antigen memory on T and B cells	Serotec[b]
CD45RA (QR, r-PE)	IgG2a	LCA for antigen naivity on T and B cells	Serotec[b]
CD25 (r-PE)	IgG1	IL-2 receptor; activated T and B cells	BD PharMingen[c]
CD71 (FITC)	IgG1	Transferrin receptor; activated T and B cells	Sigma Chemicals[a]
CD20 (r-PE)	IgG2a	B cells	Serotec[b]
CD14 (FITC)	IgG2a	monocytes	Serotec[b]
CD11c (r-PE)	IgG1	NK cells, monocytes, and macrophages.	BD PharMingen[c]
CD11b (B)	IgG1	Activated monocytes	Sigma Chemicals[a]
CD16 (r-PE)	IgG1	FcγIII receptor; NK cells, mature monocytes	BD PharMingen[c]

[a] Sigma Chemicals, St. Louis, MO
[b] Serotec, Raleigh, NC
[c] BD PharMingen, San Diego, CA

TABLE IV.1: Specificities of monoclonal antibodies used in the study. All monoclonal antibodies were purchased prelabelled to in phosphate buffered saline (pH 7.4) containing bovine serum albumin and 0.1% (w/v) sodium azide.

Abbreviations: LCA=leukocyte common antigen, IgG=immunoglobulin, FITC=fluoroscein isothiocyanate, r-PE=R-phycoerythrin, B=biotin+streptavidin conjugate, QR=quantum red, TCR=T-cell receptor, IL-2= interleukin-2, NK=natural killer cell

Table IV.2

Lymphocyte Subsets			
MAb 1	*Mab 2*	*Mab 3*	*Rationale*
CD4	CD45RO	CD25	To determine the expression of antigen exposure and naivity phenotypes and their simultaneous expression of activation markers on the CD4 cell subset
CD4	CD45RO	CD71	
CD4	CD45RA	CD25	
CD4	CD45RA	CD71	
CD8	CD45RO	CD25	To determine the expression of antigen exposure and naivity phenotypes and their simultaneous expression of activation markers on the CD8 cell subset
CD8	CD45RO	CD71	
CD8	CD45RA	CD25	
CD8	CD45RA	CD71	
CD20			To determine relative percent of B cells
Innate Immune Subsets			
CD11b	CD14	CD11c	Natural killer cells, Monocytes
CD11b	CD14	CD16	Mature monocytes

TABLE IV.2: Three-colour combinations of monoclonal antibodies (mAb) used to identify lymphocyte subsets.

Table IV.3

Post Burn	CD4	CD8	CD45RO	CD45RA	CD71	CD25	CD20	MO[1]	NK[2]
Time	(Relative percent)								
t1	38 ± 3	27 ± 2^{a}	27 ± 2^{a}	34 ± 4^{ab}	7 ± 3	17 ± 3	26 ± 3^{a}	53 ± 6^{ab}	12 ± 2^{a}
t2	43 ± 3	32 ± 2^{ab}	31 ± 2^{ab}	42 ± 4^{a}	8 ± 3	23 ± 3	23 ± 3^{a}	57 ± 6^{a}	12 ± 3^{a}
t3	43 ± 4	30 ± 3^{ab}	35 ± 3^{b}	42 ± 5^{a}	3 ± 4	20 ± 3	22 ± 3^{ab}	47 ± 6^{ab}	21 ± 5^{b}
t4	45 ± 5	29 ± 3^{ab}	32 ± 3^{ab}	32 ± 6^{ab}	13 ± 5	24 ± 4	11 ± 4^{bc}	49 ± 9^{ab}	20 ± 12^{ab}
t5	47 ± 5	35 ± 3^{b}	27 ± 3^{a}	21 ± 6^{b}	5 ± 5	22 ± 4	10 ± 4^{c}	36 ± 7^{b}	5 ± 1^{b}

[1] Percent activated macrophages were determined by the number of CD14+CD11c+ cells expressing CD16+

[2] Percent NK cells were determined by the number of CD14-CD11c+ cells

TABLE IV.3: Lymphocyte subsets were determined on whole blood deplete of red blood cells using pre-labelled immunofluorescent antibodies specific for the cell surface markers and analyzed using flow cytometry as described in the methods. Data is expressed as relative percent. Data are expressed as means ± SEM (n ≤10). Significant differences between post burn timepoints were determined using a repeated measures ANOVA and values within a column not sharing common superscripts are significantly ($p<0.05$) different.

Table IV.4

Post burn Time	*CD4*			*CD8*		
	%CD45RO	*%CD45RA*	*RO/RA Ratio*	*%CD45RO*	*%CD45RA*	*RO/RA Ratio*
t1	25.4 ± 5.1	40.1 ± 3.5	3.8 ± 0.8^{a}	41.6 ± 6.8	34.3 ± 4.6	0.8 ± 0.2
t2	36.3 ± 5.1	41.8 ± 3.5	1.3 ± 0.8^{b}	49.3 ± 6.8	28.9 ± 4.6	0.9 ± 0.2
t3	38.1 ± 6.4	37.2 ± 4.5	2.2 ± 1.0^{ab}	53.2 ± 8.6	38.9 ± 5.8	0.7 ± 0.3
t4	31.7 ± 8.5	50.2 ± 5.9	1.7 ± 1.2^{ab}	36.6 ± 11.4	27.7 ± 7.7	0.9 ± 0.3
t5	24.9 ± 7.3	42.1 ± 5.1	3.3 ± 1.0^{ab}	36.7 ± 9.8	32.2 ± 6.6	1.3 ± 0.2

TABLE IV.4: The relative percents of CD4 and CD8 cells expressing the different isotypes of the common leukocyte antigen (CD45) was determined using pre-labelled immunofluorescent monoclonal antibodies specific for the cell surface markers indicated and analyzed using flow cytometry. Data is expressed as means ± SEM (n ≤10). Significant differences between post burn timepoints were determined using a repeated measures ANOVA and values within a column not sharing common superscripts are significantly ($p<0.05$) different.

7

Table IV.5

Post Burn Time	%CD4		%CD8	
	CD25+	CD71+	CD25+	CD71+
t1	19 ± 5	17 ± 3	18 ± 5^{ab}	11 ± 3^{ab}
t2	29 ± 5	10 ± 3	20 ± 5^{a}	16 ± 3^{a}
t3	20 ± 6	8 ± 5	6 ± 7^{b}	4 ± 3^{b}
t4	26 ± 8	8 ± 5	17 ± 9^{ab}	9 ± 3^{ab}
t5	24 ± 7	7 ± 4	10 ± 8^{ab}	10 ± 3^{ab}

TABLE IV.5: The relative percents of CD4 and CD8 cells expressing the activation markers CD25 and CD71 was determined using pre-labelled immunofluorescent monoclonal antibodies specific for the cell surface markers indicated and analyzed using flow cytometry. Data is expressed as means ± SEM ($n \leq 10$). Significant differences between post burn timepoints were determined using a repeated measures ANOVA and values within a column not sharing common superscripts are significantly ($p<0.05$) different.

Table IV.6

Post Burn Time	*US*[1]	*Con A*[2]	*Con A SI*[3] *(Dpms)*	*PMA*[4]	*PMA SI*
t1	1460 ± 334^{ab}	56559 ± 8920	51 ± 8^{ab}	36951 ± 14908^{ab}	29 ± 11^{a}
t2	2107 ± 335^{a}	64152 ± 8921	33 ± 8^{a}	63704 ± 14908^{a}	44 ± 11^{ab}
t3	1302 ± 334^{ab}	48623 ± 8921	53 ± 8^{b}	65029 ± 14908^{a}	59 ± 11^{b}
t4	1496 ± 592^{ab}	37930 ± 13626	42 ± 12^{ab}	12484 ± 22772^{b}	31 ± 16^{ab}
t5	968 ± 448^{b}	56579 ± 13636	50 ± 12^{ab}	39653 ± 22772^{ab}	$40 \pm 16.^{ab}$
IL-2 production	R value	-0.11	0.81	0.76	0.66
	P value	NS	0.02	0.03	NS
IFN-γ production	R value	0.52	-0.14	0.54	0.28
	P value	0.05	NS	0.04	NS

[1] US= Unstimulated

[2] Con A=Concanavalin A

[3] SI=Stimulation Index

[4] PMA=Phorbol myristate acetate

TABLE IV.6: Proliferation of lymphocytes was measured by $[^3H]$-thymidine incorporation 66 hours before and after stimulation with Concanavalin A and phorbol myristate acetate. Values for each mitogen were correlated with the stimulated production of IL-2 and IFN-γ. Data are expressed as means ± SEM ($n \le 10$). Significant differences between post burn timepoints were determined using a repeated measures ANOVA and values within a column not sharing common superscripts are significantly ($p<0.05$) different.

Table IV.7

Fatty acid	t1	t2	t3	t4	t5
			(% fatty acids)		
18:2(6)	8.3 ± 1.0	7.5 ± 0.6	8.8 ± 1.3	10.7 ± 0.7	8.1 ± 0.9
20:2(6)	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.2	1.0 ± 0.3	0.6 ± 0.2
20:3(6)	1.3 ± 0.2^{a}	1.4 ± 0.2^{ab}	1.0 ± 0.3^{a}	1.8 ± 0.2^{ab}	2.2 ± 0.4^{b}
20:4(6)	6.6 ± 1.0^{a}	6.5 ± 0.1^{a}	7.8 ± 0.8^{ab}	8.4 ± 1.2^{ab}	10.3 ± 1.0^{b}
22:4(6)	0.6 ± 0.1	0.7 ± 0.2	0.5 ± 0.1	0.9 ± 0.1	0.6 ± 0.1
22:5(6)	0.6 ± 0.4	0.1 ± 0.1	0.7 ± 0.5	0.3 ± 0.1	0.1 ± 0.0
Σ n-6	18 ± 1^{ab}	17 ± 1^{a}	20 ± 1^{b}	21 ± 2^{ab}	20 ± 2^{ab}
18:3(3)	0.3 ± 0.1^{a}	0.1 ± 0.1^{b}	0.1 ± 0.1^{b}	0.2 ± 0.1^{ab}	0.1 ± 0.1^{ab}
18:4(3)	0.5 ± 0.2	0.2 ± 0.1	0.4 ± 0.2	0.1 ± 0.0	0.2 ± 0.1
20:5(3)	0.4 ± 0.1^{a}	0.2 ± 0.1^{b}	0.2 ± 0.1^{b}	0.2 ± 0.1^{ab}	0.3 ± 0.1^{ab}
22:5(3)	0.8 ± 0.1^{ab}	0.6 ± 0.1^{ab}	0.7 ± 0.1^{ab}	1.0 ± 0.2^{a}	0.5 ± 0.1^{b}
22:6(3)	1.5 ± 0.5	0.8 ± 0.1	0.8 ± 0.1	1.1 ± 0.2	0.8 ± 0.1
Σ n-3	4 ± 0.4^{a}	2 ± 0.4^{b}	2 ± 0.5^{b}	3 ± 1^{ab}	2 ± 1^{b}
Σ PUFA	22 ± 1^{ab}	21 ± 1^{a}	24 ± 1^{b}	24 ± 1^{b}	23 ± 1^{ab}
Σ SFA	46 ± 1^{a}	47 ± 1^{a}	46 ± 2^{a}	49 ± 2^{ab}	53 ± 2^{b}
Σ MUFA	31 ± 1^{a}	32 ± 1^{a}	30 ± 1^{ac}	28 ± 1^{bc}	26 ± 1^{b}

TABLE IV.7: Fatty acid composition of the PC fraction (isolated by thin layer chromatography) of lymphocytes at post burn timepoints expressed a percent of total fatty acids. Data are expressed as means ± SEM ($n \leq 10$). Significant differences between post burn timepoints were determined using a repeated measures ANOVA and values within a row not sharing common superscripts are significantly ($p<0.05$) different.

Table IV.8

Fatty acid	t1	t2	t3	t4	t5
	(% fatty acids)				
18:2(6)	2.9 ± 0.4	2.1 ± 0.4	2.3 ± 0.9	3.6 ± 0.7	2.6 ± 0.7
18:3(6)	0.3 ± 0.2^{ab}	0.2 ± 0.1^{ab}	1.1 ± 0.4^{a}	0.7 ± 0.4^{ab}	0.1 ± 0.1^{b}
20:2(6)	1.3 ± 1.1	0.6 ± 0.3	0.3 ± 0.1	1.3 ± 0.8	1.2 ± 0.6
20:3(6)	1.2 ± 0.2^{a}	1.8 ± 0.3^{b}	2.1 ± 0.9^{b}	1.4 ± 0.7^{ab}	2.3 ± 0.5^{b}
20:4(6)	7.9 ± 1.0^{a}	9.5 ± 1.0^{ab}	11.3 ± 1.2^{b}	12.3 ± 1.6^{b}	17.2 ± 1.4^{c}
22:3(6)	0.1 ± 0.1	0.1 ± 0.1	nil*	0.2 ± 0.2	0.2 ± 0.1
22:4(6)	0.9 ± 0.2	0.9 ± 0.2	1.2 ± 0.5	0.8 ± 0.4	1.0 ± 0.3
22:5(6)	0.2 ± 0.0	0.1 ± 0.1	0.3 ± 0.1	0.5 ± 0.5	0.2 ± 0.1
Σ n-6	15 ± 1^{a}	15 ± 1^{a}	17 ± 2^{a}	20 ± 2^{ab}	23 ± 2^{b}
18:3(3)	0.5 ± 0.4	0.1 ± 0.1	0.3 ± 0.1	0.8 ± 0.5	0.2 ± 0.1
18:4(3)	1.7 ± 1.2	0.8 ± 0.4	0.3 ± 0.1	0.1 ± 0.1	0.1 ± 1.3
20:5(3)	0.4 ± 0.2	0.2 ± 0.1	1.0 ± 0.4	0.7 ± 0.6	0.8 ± 0.6
22:5(3)	0.7 ± 0.2	0.5 ± 0.1	1.4 ± 0.6	0.3 ± 0.1	0.5 ± 0.1
22:6(3)	1.5 ± 0.3	1.0 ± 0.1	1.4 ± 0.6	0.6 ± 0.3	1.0 ± 0.2
Σ n-3	4 ± 1^{a}	3 ± 1^{b}	3 ± 1^{ab}	3 ± 1^{ab}	5 ± 1^{a}
Σ PUFA	20 ± 2^{a}	19 ± 2^{a}	24 ± 2^{ab}	25 ± 2^{ab}	26 ± 2^{b}
Σ SFA	55 ± 2^{a}	53 ± 2^{ab}	55 ± 2^{a}	55 ± 3^{ab}	48 ± 3^{b}
Σ MUFA	24 ± 2^{ab}	28 ± 2^{a}	26 ± 2^{ab}	21 ± 3^{b}	24 ± 2^{ab}

*nil=<0.04

TABLE IV.8: Fatty acid composition of the PS fraction (isolated by thin layer chromatography) of lymphocytes at post burn timepoints expressed a percent of total fatty acids. Data are expressed as means ± SEM (n ≤10). Significant differences between post burn timepoints were determined using a repeated measures ANOVA and values within a row not sharing common superscripts are significantly (p<0.05) different.

Table IV.9

Fatty Acid	t1	t2	t3	t4	t5
			(% fatty acids)		
18:2(6)	2.3 ± 0.6	3.0 ± 0.5	3.3 ± 0.5	4.5 ± 1.2	2.6 ± 0.3
18:3(6)	1.1 ± 0.9	0.1 ± 0.1	0.3 ± 0.2	0.5 ± 0.3	0.4 ± 0.3
20:2(6)	0.5 ± 0.2^{a}	0.4 ± 0.2^{a}	0.8 ± 0.6^{a}	2.4 ± 1.7^{b}	0.3 ± 0.2^{a}
20:3(6)	1.0 ± 0.2	1.8 ± 0.3	1.6 ± 0.5	2.0 ± 0.8	1.1 ± 0.4
20:4(6)	13.2 ± 2.5^{a}	22.9 ± 3.3^{b}	14.6 ± 3.4^{ab}	15.3 ± 5.6^{ab}	21.5 ± 7.1^{b}
22:3(6)	0.1 ± 0.1^{ab}	0.1 ± 0.0^{a}	0.1 ± 0.1^{ab}	0.5 ± 0.5^{b}	nil^{a*}
22:4(6)	0.3 ± 0.1	0.6 ± 0.2	0.5 ± 0.3	0.8 ± 0.1	0.5 ± 0.2
22:5(6)	0.2 ± 0.1	nil*	1.0 ± 0.8	0.3 ± 0.3	0.1 ± 0.1
Σ n-6	19 ± 3^{a}	31 ± 3^{b}	26 ± 4^{ab}	24 ± 6^{ab}	29 ± 4^{ab}
18:3(3)	0.3 ± 0.2	0.2 ± 0.1	0.4 ± 0.3	0.6 ± 0.5	0.7 ± 0.5
18:4(3)	3.1 ± 1.5	1.1 ± 0.6	1.0 ± 0.7	0.6 ± 0.6	0.1 ± 0.1
20:5(3)	2.0 ± 1.3	0.3 ± 0.1	0.3 ± 0.2	0.6 ± 0.4	0.3 ± 0.2
22:5(3)	3.1 ± 2.1	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.2	0.1 ± 0.1
22:6(3)	0.8 ± 0.3	0.5 ± 0.1	0.4 ± 0.2	0.6 ± 0.4	0.8 ± 0.4
Σ n-3	8 ± 2^{a}	2 ± 2^{b}	2 ± 2^{b}	7 ± 3^{ab}	3 ± 2^{ab}
Σ PUFA	32 ± 3	33 ± 3	29 ± 3	32 ± 5	32 ± 4
Σ SFA	12 ± 1	13 ± 1	12 ± 1	16 ± 2	13 ± 2
Σ MUFA	55 ± 3	52 ± 3	58 ± 4	53 ± 6	54 ± 5

*nil=<0.04

TABLE IV.9: Fatty acid composition of the PI fraction(isolated by thin layer chromatography) of lymphocytes at post burn timepoints expressed a percent of total fatty acids. Data are expressed as means ± SEM (n ≤10). Significant differences between post burn timepoints were determined using a repeated measures ANOVA and values within a row not sharing common superscripts are significantly ($p<0.05$) different.

Table IV.10

Fatty Acid	t1	t2	t3	t4	t5
			(% fatty acids)		
18:2(6)	4.5 ± 0.6^{a}	5.2 ± 0.7^{a}	3.2 ± 0.6^{b}	4.4 ± 0.8^{b}	3.4 ± 0.3^{b}
18:3 (6)	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1
20:2 (6)	0.5 ± 0.2^{a}	1.8 ± 0.8^{b}	0.4 ± 0.2^{a}	0.7 ± 0.1^{ab}	0.7 ± 0.1^{ab}
20:3 (6)	2.7 ± 1.9	1.7 ± 0.5	1.1 ± 0.3	1.7 ± 0.5	2.3 ± 1.0
20:4 (6)	9.2 ± 4.7^{a}	24.2 ± 4.3^{b}	22.7 ± 4.7^{ab}	20.8 ± 7.7^{ab}	30.9 ± 5.8^{b}
22:3 (6)	nil*	nil*	nil*	0.1 ± 0.1	0.1 ± 0.1
22:4 (6)	1.8 ± 0.6	3.1 ± 1.0	3.2 ± 1.0	4.9 ± 2.2	4.6 ± 1.1
22:5 (6)	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.5 ± 0.1	0.3 ± 0.1
Σ n-6	23 ± 4^{a}	33 ± 3^{ab}	33 ± 4^{ab}	30 ± 7^{ab}	38 ± 5^{b}
18:3 (3)	0.4 ± 0.2^{a}	0.4 ± 0.2^{ab}	0.7 ± 0.2^{ab}	0.6 ± 0.4^{ab}	1.0 ± 0.2^{b}
18:4 (3)	0.9 ± 0.3	0.3 ± 0.1	0.6 ± 0.4	0.8 ± 0.8	0.22 ±
20:5 (3)	0.3 ± 0.2^{a}	0.3 ± 0.3^{a}	0.5 ± 0.3^{ab}	0.4 ± 0.4^{ab}	1.4 ± 0.3^{b}
22:5 (3)	1.6 ± 0.5	2.0 ± 0.4	1.8 ± 0.4	1.9 ± 0.5	1.9 ± 0.5
22:6 (3)	1.7 ± 0.5	1.7 ± 0.4	2.3 ± 0.8	2.0 ± 0.3	1.8 ± 0.6
Σ n-3	8 ± 1	5 ± 1	6 ± 1	7 ± 4	6 ± 2
Σ PUFA	34 ± 3^{a}	14 ± 3^{ab}	45 ± 3^{b}	37 ± 7^{ab}	44 ± 4^{ab}
Σ SFA	45 ± 3^{a}	34 ± 3^{b}	37 ± 3^{b}	45 ± 6^{ab}	41 ± 4^{ab}
Σ MUFA	19 ± 1^{ab}	22 ± 1^{a}	19 ± 1^{ab}	18 ± 3^{ab}	16 ± 2^{b}

*nil=<0.04%

TABLE IV.10: Fatty acid composition of the PE fraction (isolated by thin layer chromatography) of lymphocytes at post burn timepoints expressed a percent of total fatty acids. Data are expressed as means ± SEM (n ≤10). Significant differences between post burn timepoints were determined using a repeated measures ANOVA and values within a row not sharing common superscripts are significantly ($p<0.05$) different.

Table IV.11

Post burn Time	*PE*	*PC*	*PS*	*PI*
	(18:2n-6/20:4n-6 Ratio)			
t1	0.7 ± 0.1	1.6 ± 0.2^{a}	0.8 ± 0.1^{a}	0.3 ± 0.1
t2	0.8 ± 0.1	1.4 ± 0.2^{ab}	0.3 ± 0.1^{b}	0.3 ± 0.1
t3	0.6 ± 0.1	1.1± 0.2^{ab}	0.4 ± 0.2^{ab}	0.3 ± 0.1
t4	0.7 ± 0.2	1.0 ± 0.3^{ab}	0.1 ± 0.2^{b}	0.6 ± 0.2
t5	0.5 ± 0.2	0.8 ± 0.3^{b}	0.1 ± 0.2^{b}	0.2 ± 0.2

TABLE IV.11: The 18:2n-6/20:4n-6 ratio in major PL fractions (separated by thin layer chromatography) of lymphocytes isolated at specific times during recovery from burn injury was determined using GC analysis. Data are expressed as means ± SEM ($n \leq 10$). Differences between post burn timepoints were determined using a repeated measures ANOVA and values within a column not sharing common superscripts are significantly ($p<0.05$) different.

E. References

Akbar AN, Salmon M, Janossy G. The synergy between naive and memory T cells during activation. Immunol Today 12: 184-188, 1991.

Alexander JW. William A. Altemeier lecture. Nutrition and infection. New perspectives for an old problem. Arch Surg 121: 966-972, 1986.

Bancroft GJ. The role of natural killer cells in innate resistance to infection. Curr Opin Immunol 5: 503-510, 1993.

Barlow Y. T lymphocytes and immunosuppression in the burned patient: a review. Burns 20: 487-490, 1994.

Birke G, Carlson LA, Liljedahl SO. Lipid metabolism and trauma. 3. Plasma lipids and lipoproteins in burns. Acta Med Scand 178: 337-350, 1965.

Blazar BA, Rodrick ML, O'Mahony JB et al. Suppression of natural killer-cell function in humans following thermal and traumatic injury. J Clin Immunol 6: 26-36, 1986.

Blok WL, Katan MB, van der Meer JW. Modulation of inflammation and cytokine production by dietary (n- 3) fatty acids. J Nutr 126: 1515-1533, 1996.

Bower RH, Cerra FB, Bershadsky B et al. Early enteral administration of a formula (Impact) supplemented with arginine, nucleotides, and fish oil in intensive care unit patients: results of a multicenter, prospective, randomized, clinical trial. Crit Care Med 23: 436-449, 1995.

Braquet M, Lavaud P, Dormont D et al. Leukocytic functions in burn-injured patients. Prostaglandins 29: 747-764, 1985.

Calder PC. Dietary fatty acids and the immune system. Lipids 34 Suppl: S137-S140, 1999.

Carter EA and Tompkins RG. Injury-induced inhibition of fat absorption. J Burn Care Rehab 15: 154-157, 1994.

Cerra FB. Nutrient modulation of inflammatory and immune function. Am J Surg 161: 230-234, 1991.

Cerra FB, Lehmann S, Konstantinides N et al. Improvement in immune function in ICU patients by enteral nutrition supplemented with arginine, RNA, and menhaden oil is independent of nitrogen balance. Nutrition 7: 193-199, 1991.

Cetinkale O and Yazici Z. Early postburn fatty acid profile in burn patients. Burns 23: 392-399, 1997.

Chan SH, Kobayashi M, Santoli D et al. Mechanisms of IFN-gamma induction by natural killer cell stimulatory factor (NKSF/IL-12). Role of transcription and mRNA stability in the synergistic interaction between NKSF and IL-2. J Immunol 148: 92-98, 1992.

Chang KJ, Saito H, Tatsuno I et al. Comparison of the effect of lipoxygenase metabolites of arachidonic acid and eicosapentaenoic acid on human natural killer cell cytotoxicity. Prost Leukot Essent Fatty Acids 38: 87-90, 1989.

Clement LT. Isoforms of the CD45 Common Leukocyte antigen family: Markers for Human T-cell differentiation. J Clin Immunol 12: 1-10, 1992.

Coombes EJ, Shakespeare PG, Batstone GF. Lipoprotein changes after burn injury in man. J Trauma 20: 971-975, 1980.

Daly JM, Redmond HP, Gallagher H. Perioperative nutrition in cancer patients. JPEN J Parenter Enteral Nutr 16: 100S-105S, 1992.

de Pablo MA and Alvarez de Cienfuegos G. Modulatory effects of dietary lipids on immune system functions. Immunol Cell Biol 78: 31-39, 2000.

de Pablo M., Ortego E, Gallego AM et al. The effect of dietary fatty acid manipulation on phagocytic actvity and cytokine production by peritoneal macrophages from Balb/c mice. J Nutr Sci Vitaminol 44:67, 1998

Faist E, Schinkel C, Zimmer S et al. Inadequate interleukin-2 synthesis and interleukin-2 messenger expression following thermal and mechanical trauma in humans is caused by defective transmembrane signalling. J Trauma 34: 846-854, 1993.

Field CJ, Gougeon R, Marliss EB. Circulating mononuclear cell numbers and function during intense exercise and recovery. J Appl Physiol 71: 1089-1097, 1991.

Fukushima R, Alexander JW, Wu JZ et al. Time course of production of cytokines and prostaglandin E2 by macrophages isolated after thermal injury and bacterial translocation. Circ Shock 42: 154-162, 1994.

Gadd MA, Hansbrough JF, Hoyt DB et al. Defective T-cell surface antigen expression after mitogen stimulation. An index of lymphocyte dysfunction after controlled murine injury. Ann Surg 209: 112-118, 1989.

Gerster H. The use of n-3 PUFAs (fish oil) in enteral nutrition. Int J Vitam Nutr Res 65: 3-20, 1995.

Gottschlich MM and Alexander JW. Fat kinetics and recommended dietary intake in burns. JPEN J Parenter Enteral Nutr 11: 80-85, 1987.

Gottschlich MM and Warden GD. Vitamin supplementation in the patient with burns. J Burn Care Rehab 11: 275-279, 1990.

Grbic JT, Mannick JA, Gough DB et al. The role of prostaglandin E2 in immune suppression following injury. Ann Surg 214: 253-263, 1991.

Jeffery NM, Sanderson P, Sherrington EJ et al. The ratio of n-6 to n-3 polyunsaturated fatty acids in the rat diet alters serum lipid levels and lymphocyte functions. Lipids 31: 737-745, 1996.

Kelley DS and Daudu PA. Fat intake and immune response. Prog Food Nutr Sci 17: 41-63, 1993.

Kemen M, Senkal M, Homann HH et al. Early postoperative enteral nutrition with arginine-omega-3 fatty acids and ribonucleic acid-supplemented diet versus placebo in cancer patients: an immunologic evaluation of Impact. Crit Care Med 23: 652-659, 1995.

Kinsella JE and Lokesh B. Dietary lipids, eicosanoids, and the immune system. Crit Care Med 18: S94-113, 1990.

Lightstone L and Marvel J. CD45RA+ T cells: not simple virgins. Clin Sci (Colch) 85: 515-519, 1993.

Lokesh B, LiCari J, Kinsella JE. Effect of different dietary triglycerides on liver fatty acids and prostaglandin synthesis by mouse peritoneal cells. JPEN J Parenter Enteral Nutr 16: 316-321, 1992.

Lyons A, Kelly JL, Rodrick ML et al. Major injury induces increased production of interleukin-10 by cells of the immune system with a negative impact on resistance to infection. Ann Surg 226: 450-468, 1997.

Markovic SN and Murasko DM. Anesthesia inhibits interferon-induced natural killer cell cytotoxicity via induction of CD8+ suppressor cells. Cell Immunol 151: 474-480, 1993.

Mosmann TR and Sad S. The expanding universe of T-cell subsets: Th1, Th2 and more. Immunol Today 17: 138-146, 1996.

O'Riordain DS, Mendez MV, Holzheimer RG et al. Interleukin-2 receptor expression and function following thermal injury. Arch Surg 130: 165-170, 1995.

O'Sullivan ST, Lederer JA, Horgan AF et al. Major injury leads to predominance of the T helper-2 lymphocyte phenotype and diminished interleukin-12 production associated with decreased resistance to infection. Ann Surg 222: 482-492, 1995.

O'Sullivan ST and O'Connor TP. Immunosuppression following thermal injury: the pathogenesis of immunodysfunction. Br J Plast Surg 50: 615-623, 1997.

Ogle CK, Guo X, Wu JZ et al. Production of cytokines and PGE2 and cytotoxicity of stimulated bone marrow macrophages after thermal injury and cytotoxicity of stimulated U-937 macrophages. Inflamm 17: 583-594, 1993.

Ogle CK, Wu JZ, Wood S et al. The increased release of prostaglandin E2 by Kupffer cells from burned guinea pigs. J Burn Care Rehab 11: 287-294, 1990.

Oxholm P, Pedersen BK, Horrobin DF. Natural killer cell functions are related to the cell membrane composition of essential fatty acids: differences in healthy persons and patients with primary Sjogren's syndrome. Clin Exp Rheumatol 10: 229-234, 1992.

Pollack R, Lotzova E, Stanford S. Mechanism of surgical stress impairment on human perioperative natural killer cell cytotoxicity. Arch Surg 126: 338-342, 1991.

Romagnani S. Biology of human TH1 and TH2 cells. J Clin Immunol 15: 121-129, 1995.

Saffle JR, Wiebke G, Jennings K et al. Randomized trial of immune-enhancing enteral nutrition in burn patients. J Trauma 42: 793-800, 1997.

Sayeed MM. Alterations in cell signaling and related effector functions in T lymphocytes in burn/trauma/septic injuries. Shock 5: 157-166, 1996.

Schluter B, Konig B, Bergmann U et al. Interleukin 6--a potential mediator of lethal sepsis after major thermal trauma: evidence for increased IL-6 production by peripheral blood mononuclear cells. J Trauma 31: 1663-1670, 1991a.

Schluter B, Konig W, Koller M et al. Differential regulation of T- and B-lymphocyte activation in severely burned patients. J Trauma 31: 239-246, 1991b.

Schultz RM. Autocrine versus lymphocyte-dependent mechanisms for macrophage activation. Cell Signal 3: 515-522, 1991.

Seaman WE and Woodcock J. Human and murine natural killer cell activity may require lipoxygenation of arachidonic acid. J Allergy Clin Immunol 74: 407-411, 1984.

Singh H, Abdullah A, Herndon DN. Effects of rat interleukin-2 and rat interferon on the natural killer cell activity of rat spleen cells after thermal injury. J Burn Care Rehab 13: 617-622, 1992.

Tebbey PW and Buttke TM. Arachidonic acid regulates unsaturated fatty acid synthesis in lymphocytes by inhibiting stearoyl-CoA desaturase gene expression. Biochim Biophys Acta 1171: 27-34, 1992.

Teodorczyk-Injeyan JA, Sparkes BG, Mills GB et al. Immunosuppression follows systemic T lymphocyte activation in the burn patient. Clin Exp Immunol 85: 515-518, 1991.

Teodorczyk-Injeyan JA, Sparkes BG, Mills GB et al. Impairment of T cell activation in burn patients: a possible mechanism of thermal injury-induced immunosuppression. Clin Exp Immunol 65: 570-581, 1986.

Tomita-Yamaguchi M, Rubio C, Santoro TJ. Regional influences on the physical properties of T cell membranes. Life Sci 48: 433-438, 1991.

Traill KN and Wick G. Lipids and Lymphocyte Function. Immunol Today 5: 20-76, 1986.

Tripp CS, Wolf SF, Unanue ER. Interleukin 12 and tumor necrosis factor alpha are costimulators of interferon gamma production by natural killer cells in severe combined immunodeficiency mice with listeriosis, and interleukin 10 is a physiologic antagonist. Proc Natl Acad Sci USA 90: 3725-3729, 1993.

Vega GL and Baxter CR. Induction of hypertriglyceridemia in rabbits by thermal injury: I. Time course of elevated plasma triglyceride levels. J Burn Care Rehab 9: 266-270, 1988.

Wallace FA, Yaqoob P, Miles EA et al. Dietary fat influences the production of Th1- but not Th2-derived cytokines. Lipids 34 Suppl: S141, 1999.

Waymack JP, Guzman RF, Burleson DG et al. Effect of prostaglandin E in multiple experimental models. VI. Effect on T-cell subsets. Prostaglandins 38: 345-353, 1989.

Weimann A, Bastian L, Bischoff WE et al. Influence of arginine, omega-3 fatty acids and nucleotide-supplemented enteral support on systemic inflammatory response syndrome and multiple organ failure in patients after severe trauma. Nutrition 14: 165-172, 1998.

Wood JJ, Rodrick ML, O'Mahony JB et al. Inadequate interleukin 2 production. A fundamental immunological deficiency in patients with major burns. Ann Surg 200: 311-320, 1984.

Wu D, Meydani SN, Meydani M et al. Immunologic effects of marine- and plant-derived n-3 polyunsaturated fatty acids in nonhuman primates. Am J Clin Nutr 63: 273-280, 1996.

Yaqoob P, Newsholme EA, and Calder PC. The effect of dietary lipid manipulation on rat lymphocyte subsets and proliferation. Immunol 82:603-610, 1994

V.

Fatty Acid Content of Plasma Lipids and Erythrocyte Phospholipids are Altered Following Burn Injury

A. Introduction

There are profound alterations in fatty acid metabolism following burn injury (Gottschlich et al.,1987; Wolfe et al.,1983). Fatty acid oxidation rates, free fatty acid turnover and lipolysis are elevated (Barton,1994; Wolfe et al.,1987). In addition, plasma levels of 18:2n-6 (linoleic acid) and 20:4n-6 (arachidonic acid) have been reported to be reduced (Harris et al.,1981) while SFA and oleic acid are increased, characteristics observed in essential fatty acid deficiency (Barton,1994; Gottschlich et al., 1987). Diet is an important part of therapy for burn patients and lipids must be included in the diet to provide energy, aid in the absorption of fat soluble vitamins and prevent essential fatty acid deficiency. Essential fatty acids are involved in wound healing (Caffrey et al.,1981; Hulsey et al.,1980) and serve important roles in immune functions (Calder,1995; de Pablo et al.,2000). Dietary lipids have profound effects on immune functions and inflammatory processes through changing cellular membranes and altering substrate availability for the synthesis of lipid mediators such as prostaglandins (Alexander,1986; Calder,1999; Fischer et al.,1991; Gottschlich,1992; Kinsella et al.,1990) that may predispose patients to infection. Preventing infection and reducing inflammatory processes are important goals in the treatment of burn patients. Before diets designed to improve immune recovery in these patients can be formulated, an understanding of the metabolism of specific fatty acids, particularly the essential fatty acids involved in wound healing and immunological functions following burn injury is needed. The objective of this study was to characterize quantitative and compositional changes in fatty acids in plasma components and red blood cells immediately following and during recovery from burn injury.

A version of this chapter has been submitted for publication.
Pratt et al., 2001. Lipids.

B. Methods

Materials, subjects and sample collection for this study were described in Section III.B.2.

1. Serum Collection

Whole blood (3 ml) was layered on top of ficoll hypaque gradients and centrifuged as previously described in Section III.6. Plasma was collected from the top and stored immediately at -70°C for plasma lipid analysis. After gradients were removed, remaining red blood cells were washed once with KRH buffer with BSA (5 g/L). Red blood cells were frozen immediately for fatty acid analysis of phospholipids.

2. Fatty Acid Analysis

a) *Lipid Extraction*

Previously frozen plasma from the top of the gradient (described above) was thawed and 100 μl transferred to a glass tube. Lipids were extracted from the sample using chloroform/methanol (2:1 by volume) and the sample sat overnight at 4°C. The lower phase containing the lipids was removed and transferred to a screw cap tube using a glass pipet and dried down under nitrogen gas. Chloroform (100 μl) was added to the dried sample.

b) *Thin Layer Chromatography*

Lipid classes were separated using "G" plates for total phospholipids (PL), cholesteryl esters (CE) and triglycerides (TG) and "H" plates for PC, PE, and PI in plasma. Plates were heat activated for 1 hour prior to spotting the plates with 50 μl of the extracted lipid. Solvent tanks were lined with Watman #1 fliter paper and saturated with the solvent systems for 1 hour before plates were placed in the tank. "G" plates were processed using a solvent system using petroleum ether/diethyl ether/glacial acetic acid (80:20:1 by volume) until samples reached the top. "H" plates were processed in a solvent system using chloroform / methanol / 2-propanol / 0.25% KCl/triethylamine (30:9:25:6:18 by volume). Plates were air dried for 2-3 minutes, sprayed with ANSA and bands visualized by UV light. The PL, CE and TG fractions on the G-plates and the PI, PC and PE on the H-plates were identified and placed in methylation tubes. Standards were added to the PL (10 μg C17:0), TG (20 μg C15:0) and CE (20 μg C15:0) bands.

c) *Saponification*

Methanolic KOH (500 μl of 0.5 N) was added to the TG and CE samples and tubes heated in a hot sand bath at 110°C for 1 and 1.5 hours respectively followed by direct methylation.

d) *Methylation*

Hexane (2 ml) and BF_3 in methanol (1.5 ml) was added to the samples. Samples were capped and heated at 100-110°C in a coarse sand bath for 1 hour. After samples cooled, 2 ml of distilled water was added, tubes vortexed and placed at 4°C overnight. The upper layer containing the fatty acid methyl esters was removed and placed in a GC vial and dried down under nitrogen.

e) *Gas-Liquid Chromatography*

Fatty acid methyl esters were separated by an automated gas-liquid chromatograph (GLC), Varian model 6000 equipped with a Vista 654 data system and a Vista 8000 autosampler (Varian Instrument Company, Georgetown Ontario). The system used a bonded phase fused silica capillary column (100 m x 0.25 mm i.d x 0.2 μm film thickness; Supelco, Inc., Bellefonte, PA). Helium was used as the carrier gas at a flow rate of 1.8 ml/minute using a splitless injector. The GLC oven temperature was programmed for a 2 stage increase from an initial temperature of 150°C to 190°C at 20°C/minute and held for 23 minutes followed by a second stage temperature increase to 220°C at 2°C /minute for a total analysis time of 40 minutes. Peaks of fatty acid methyl esters were identified by comparisons with authentic compounds purchased from Supelco Canada (Bellefonte, PA) and Sigma Chemical (St. Louis, MO) companies. These GLC operating conditions are capable of separating methyl esters of all saturated, *cis*-monounsaturated and *cis*-polyunsaturated fatty acids for 14-24 carbon chains in length. Fatty acid content was calculated from the internal standard added.

Fatty acid content of plasma lipid classes were calculated using the area peak of the internal standard. PL, CE, TG and their respective contents of saturated, monounsaturated, polyunsaturated as well as the essential fatty acids and their elongation/desaturation products were calculated on both a quantitative and percent basis. Percent fatty acids in red blood cell PL and major PL in serum were determined.

3. Statistical Analysis

Data is reported as means ± SEM. Subjects were grouped into groups based on size of burn (large= >35%, n=5 and small=<35%, n=5) and when differences existed between these two groups, results are presented. When there were no differences between the two burn injury groups, all subjects were included in the analysis and overall mean presented. To determine differences between post burn timepoints, a repeated measures analysis of variance was used to identify differences in fatty acids in each plasma fraction at the 5 post burn time periods described in Section III.B.9 (t1=0-12 d; t2=13-19d; t3=20-36 d; t4=37-49 d and t5=>50 days). Number of subjects included in the analysis at each timepoint was as follows: t1, n=10; t2, n=10; t3, n=8; t4, n=6; t5, n=8. Subjects with burns >35% TBSA were sampled at every timepoint. When significant differences were identified, differences between time periods were identified using least square means. A t-test was used to identify differences between post burn timepoints and control values. All statistical analyses were conducted using the SAS statistical package (Version 6.12, SAS Institute, Cary, NC).

C. Results

1. Dietary Intake

Dietary intakes of n-6 and n-3 fatty acids are shown in Table III.1.

2. Plasma Total Phospholipids

Percent of 20:4n-6 and total n-6 in plasma phospholipids were significantly lower at t1 compared to t5 (Table V.1). Although not different on a relative percent basis, quantitatively, the n-3 fatty acid content was lower at t1 and t2 than at t5 (Table V.2). The total percent of MUFA in plasma PL was higher at t1 compared to t3, t4 and t5 (Table V.1). Compared to healthy control subjects, the relative proportion of total n-6 fatty acid was lower and SFA higher at all post burn timepoints (Table V.1). At t1 and t2, plasma PL exhibited lower percent of 20:4n-6 and higher proportion of MUFA compared to healthy control subjects. There were no significant differences in total plasma PL concentration between post burn timepoints however, at t1 and t2, the total plasma PL concentration was significantly less than healthy controls (Figure V.1 and Table V.1). The complete fatty acid profile of plasma PL following burn injury is shown in Appendix V.AP1 and that of healthy individuals in Appendix V.AP4.

3. Major Phospholipid Fractions

There was no significant difference in the 20:4n-6 content in the major plasma PL fractions (PC=6.7 ± 0.9%; PE=14.8 ± 1.8%; PI= 6.9 ± 1.6%) between post burn timepoints, however, PE and PI fractions contained significantly less 20:4n-6 than control subjects (PE=20.2 ± 2.2%; PI=22.9 ± 1.9%; n=6) at t1 and t2, and t1-t3 post burn timepoints, respectively (data not illustrated). In the PI fraction there was a significant increase in total n-6 fatty acids and a decrease in n-3 fatty acid content at t2 compared to t1 (Table V.3). SFA content of the PI and PE fractions was significantly greater at t1 and t3 (PI= 47.5 ± 1.3%; PE= 40.9 ± 1.4%) as compared to control subjects (PI= 41.3 ± 2.3%; PE= 34.3 ± 1.5%). At all post burn timepoints, PC and PE contained less percent n-3 fatty acids than controls (Table V.3).

4. Cholesteryl Esters

Proportions of 20:4n-6 and total n-6 fatty acids in plasma CE were significantly lower at t1 and t2 compared to t5 (Table V.4). CE isolated at t5 contained significantly more n-3 fatty acids than any other post burn timepoint (Table V.4, Table V.5). The percent of SFA was higher at t1 and t2 compared to t5. On a percent basis, CE isolated from burn patients at t1, t2 and t3 contained significantly less n-6 fatty acids than control subjects (Table V.4). The percent SFA was higher than that of control subjects at sample points t1-t4. At every post burn timepoint there was a lower concentration of CE in plasma (Table V.5 and Figure V.2) and significantly ($p<0.05$) lower amounts of 20:4n-6, n-6 and n-3 fatty acids (Table V.5) compared to control values (data not illustrated). The complete fatty acid profile of plasma CE following burn injury is shown in Appendix V.AP2 and that of healthy individuals in Appendix V.AP4.

5. Triglycerides

The total percent n-6 fatty acids in plasma TG was lower at t1 than at t4 and t5 (Table V.6). Total n-3 fatty acid content was lower ($p<0.05$) at t2 compared to t5. Quantitatively, 20:4n-6 content at t1 (4.5 ± 0.9 μg/ml) was less than that of control subjects (8.5 ± 1.4 μg/ml). The n-3 fatty acid content at the first 4 timepoints (6.3 ± 0.9 μg/ml) was half that of control subjects (15.5 ± 2.6 μg/ml, data not illustrated). The complete fatty acid profile of plasma TG following burn injury is shown in Appendix V.AP3 and that of healthy individuals in Appendix V.AP4.

6. Erythrocyte Phospholipids

The content of major n-6 and n-3 fatty acids for PC and PE, the major PL of RBCs, during the post burn period are depicted in Table V.7 and reference ranges of healthy adults reported in the literature are shown. In the PC (Table V.7A), PS (Table V.9) and PI (Table V.10) phospholipid fractions of RBC, the relative percent of 20:4n-6 was significantly lower at t1 than at t5. Every RBC PL fraction exhibited half the levels of n-3 fatty acids of reference values at all post burn timepoints (data shown for PC and PE only in Tables 7A and 7B). Complete fatty acid profiles of all RBC PL fractions are shown in Appendices V.AP4-AP8.

D. Discussion

The results of this study support the hypothesis that essential fatty acid metabolism is altered following burn injury. This study confirms other reports of reduced PL and CE levels following burn injury (Batstone et al.,1976; Birke et al.,1965; Coombes et al.,1979; Coombes et al.,1980; Harris et al., 1981; Kaufman et al.,1978) and expands existing knowledge by demonstrating that n-6, particularly 20:4n-6, and n-3 fatty acids are reduced in the early post burn period in plasma and erythrocytes. Reduced amounts of essential fatty acids in plasma suggest decreased availability from the diet or an increase in their utilization by the tissues.

A diet high in linoleic acid (18:2n-6) in healthy subjects would be expected to increase n-6 fatty acids in plasma (James et al.,1993). However, patients in this study exhibited lower n-6 and 20:4n-6 content early after burn injury when 18:2n-6 intake from the enteral diet was high. Despite the return of total plasma PL concentrations to values not different from healthy controls by t3, the content and relative percent of n-6 and total PUFA in plasma remained low throughout the study. Low levels of 18:2n-6 and 20:4n-6 in total plasma lipid have previously been reported following burn injury (Harris et al.,1982) and this report confirms that this occurs in each of the lipid fractions that comprise the total plasma lipid pool. Low levels of 20:4n-6 in plasma PL and CE may suggest increased utilization of 20:4n-6 during the early post burn period. Prostaglandins (PG) derived from 20:4n-6 have been reported to be increased post burn (Fukushima et al.,1994; Waymack et al.,1989) and have been implicated in immunosuppression associated with burn injury (Grbic et al.,1991; Teodorczyk-Injeyan et al.,1987). Fatty acids in plasma have been reported to exchange with immune cell membranes (James et al., 1993) and could potentially impact on PG synthesis and wound healing (Hulsey et al.,1977).

N-3 fatty acid content of each plasma fraction was low in the early post burn period and in some cases, persisted even 50 days after the initial injury. Reductions in the content of n-3 fatty acids observed in plasma TG (primarily from chylomicrons and VLDL fractions) suggest reduced availability to tissues. N-3 fatty acids are important in balancing the production of cyclooxygenase products derived from 20:4n-6 (Gerster,1995) and have also been demonstrated to reduce inflammatory responses (Blackburn,1992; Caughey et al.,1996; de Pablo et al., 2000). Increased SFA and MUFA were observed in all plasma fractions which is consistent with increased lipolysis in these patients (Cetinkale et al.,1997; Smith et al.,1983)

Quantitative and compositional differences between patients and control subjects in this study suggest impairments in fatty acid assimilation and/or lipoprotein metabolism. It is unknown if the decrease in plasma components hinder utilization of essential fatty acids by tissues or if there is increase tissue utilization causing depletion in the plasma. Lipoproteins differ in CE, TG and PL constituents (Groff et al.,1995). Marked reductions in plasma concentrations of CE and PL early after burn observed in this study and supported by others (Batstone et al., 1976; Birke et al., 1965; Coombes et al., 1979; Coombes et al., 1980; Harris et al., 1981; Kaufman et al., 1978) suggest a change in the relative proportions of the individual lipoprotein fractions or an overall reduction in their synthesis. Unfortunately, lipoprotein fractions were not isolated in the present study. Concentrations of LDL and HDL have been reported to be reduced following burn injury (Birke et al., 1965; Coombes et al., 1980) which would proportionately reduce cholesterol and PL in the plasma. Deficiencies in intestinal lipases (Carter et al.,1994), lipoprotein carrier proteins (Vega et al.,1988), carnitine (Harris et al., 1982) and liver functions (Aarsland et al.,1996; Birke et al., 1965; Czaja et al.,1975; Huang et al.,1998; Mittendorfer et al.,1998) have been reported following burn injury and might contribute to altered lipid metabolism and the compositional and quantitative changes observed in the plasma lipid components in this study.

The fatty acid composition of red blood cells is frequently used as a method to estimate the composition of dietary fat and abnormalities in essential fatty acid metabolism (Brown et al.,1991; Stanford et al.,1991). It is likely that a sufficient amount of 18:2n-6 was provided by the diet as 18:2n-6 levels were relatively constant throughout the study and were similar to control values with the exception of a transient decrease at t2 in the PC fraction. In spite of this, all RBC PL fractions exhibited lower 20:4n-6 levels than reference values throughout the post burn period (Agren et al.,1995; Innis,1988). These observations are inconsistent with Diboune (Diboune et al.,1992) who observed increases in 18:2n-6 at 14 days and no change in 20:4n-6 levels when intensive care patients were provided an enteral diet with similar MCT and 18:2n-6 content as the enteral diet provided in this study. Despite dietary intake consisting of higher amounts of PUFA than MUFA and SFA (apart from the rapidly oxidized MCT), RBCs exhibited higher SFA and MUFA than PUFA in PL early after burn and compared to control subjects. These findings parallel the observations in the various plasma components and are consistent with essential fatty acid deficiencies in the early post burn period (Gottschlich et al., 1987). Low concentrations of 20:4n-6, 22:6n-3 and 18:2n-6 with high levels of SFA have been reported in total RBC lipids following burn injury (Harris et al., 1981). In the current study, 22:6n-3 levels remained lower than healthy controls at all post burn timepoints. If subjects were provided adequate amounts of 18:2n-6, slow conversion to 20:4n-6 is a possible explanation of low 20:4n-6 levels observed in all measured RBC PL fractions and plasma components. Reduced delta-6-desaturase activity is supported by the observations of low levels of 22:5n-3 and 22:6n-3 and 20:3n-6 at t1 compared to t5 (and controls) even though 18:3n-3 content of RBCs was similar to healthy controls and exhibited little change throughout the post burn period. This pathway is reported to be reduced by a large essential fatty acid intake (Blond et al.,1984; Innis,1986; Voss et al.,1988) and with burn injury (Brenner,1981). Several functions of RBCs have been reported to be altered following burn injury (Deitch et al.,1993; Shen et al.,1943; Terry et al.,1975; Welt,1967), some of which are known to be affected by PL composition of the membrane (Kahlenberg et al.,1972; Kinsella,1990).

This study demonstrates essential fatty acid levels to be reduced in both plasma and RBCs following burn injury and points to deficiencies in key enzymes involved in regulation of lipid metabolism. All lipid containing plasma components measured exhibited lower n-6 and n-3 fatty acid content and higher SFA and MUFA content in early post burn timepoints compared to later post burn timepoints and those of control subjects. Decreased essential fatty acids in plasma may be suggestive of the increased use of these lipids in the synthesis of membrane lipids for wound healing and immune functions. Achieving the optimal balance of fatty acids for skin regeneration, immune competence and inflammatory processes through provision of specific fatty acids through diet is a complex issue. Further work is needed to determine the ability of burn patients to utilize essential fatty acids and to design nutritional intervention that prevents fatty acid deficiencies early after burn while maintaining optimal wound healing and immunological functions consistent with recovery in these patients.

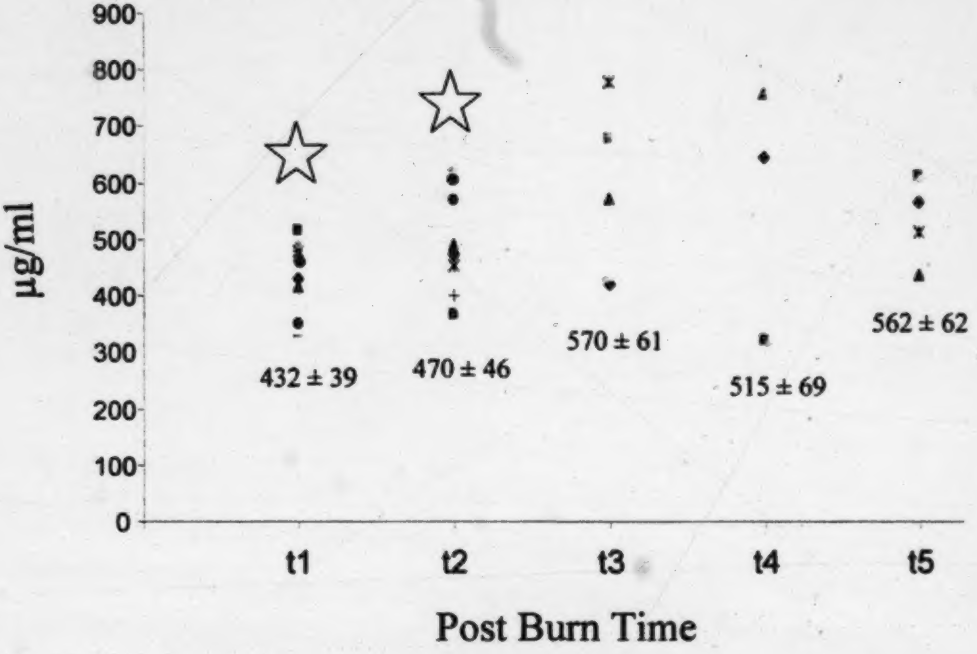


FIGURE V.1: Total plasma PL of subjects ($n \leq 10$). Data points represent values from individual patients at the post burn times indicated. Means are shown numerically. Shaded region represents values of healthy controls (mean ± SEM, n=8). Significant differences between post burn timepoints were determined using a repeated measures ANOVA and values not sharing common superscripts are significantly different ($p<0.05$). Timepoints with a star indicates a significant difference ($p<0.0.5$) from healthy subjects (reference; n=6).

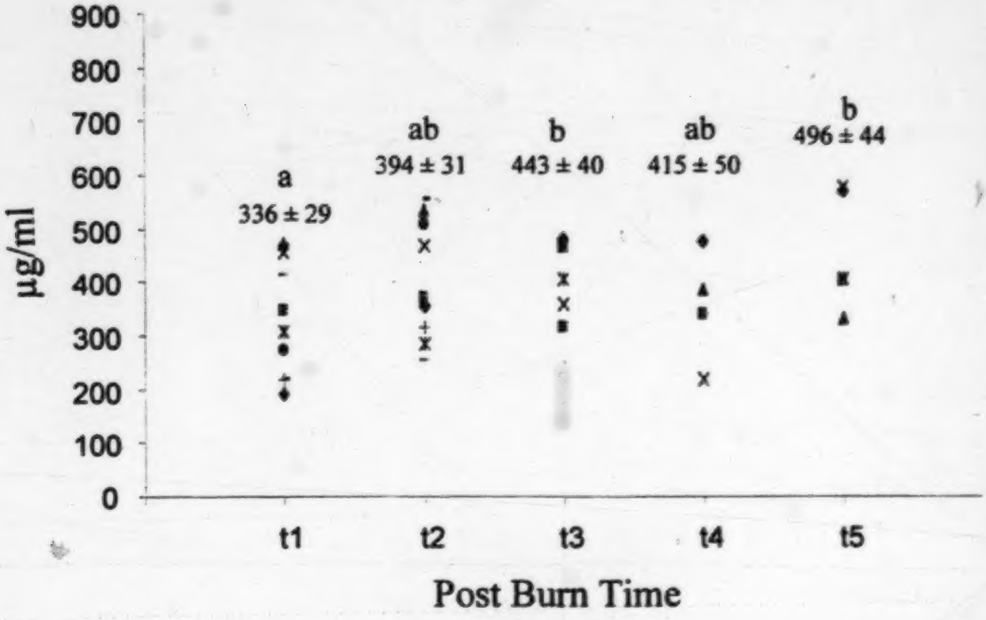


FIGURE V.2: Total plasma CE of subjects (n ≤10). Data points represent values from individual patients at the post burn times indicated. Means are shown numerically. Shaded region represents values of healthy controls (mean ± SEM). Significant differences during recovery were determined using a repeated measures ANOVA and values not sharing common superscripts are significantly different ($p<0.05$). At all timepoints, the concentration of CE was significantly ($p<0.05$) less than healthy subjects (reference; n=6).

Table V.1

Post Burn Time	*20:4n-6*	*Σn-6*	*Σn-3*	*ΣSFA*	*ΣMUFA*	*Total (μg/ml)*
	(% of total fatty acids)					
t1	$6.9 \pm 0.5^{a*}$	$27.8 \pm 0.9^{a*}$	3.8 ± 0.3	$49.0 \pm 0.5^{a*}$	$18.7 \pm 0.7^{a*}$	$432.4 \pm 39.0^{*}$
t2	$7.4 \pm 0.5^{ab*}$	$30.1 \pm 1.0^{ab*}$	3.5 ± 0.3	$49.2 \pm 0.5^{ab*}$	$16.6 \pm 0.8^{ab*}$	$470.4 \pm 46.1^{*}$
t3	8.3 ± 0.7^{ab}	$30.3 \pm 1.3^{ab*}$	3.6 ± 0.4	$50.5 \pm 0.7^{ab*}$	15.5 ± 1.0^{b}	569.9 ± 61.1
t4	8.2 ± 0.8^{ab}	$28.9 \pm 1.5^{ab*}$	3.6 ± 0.4	$51.7 \pm 0.8^{b*}$	15.2 ± 1.2^{b}	515.0 ± 69.1
t5	8.7 ± 0.7^{b}	$31.5 \pm 1.4^{b*}$	3.6 ± 0.4	$50.7 \pm 0.7^{b*}$	14.2 ± 1.0^{b}	551.5 ± 61.5
Reference	*9.1 ±0.6*	*35.6 ±1.2*	*3.8 ±0.3*	*45.6 ±1.0*	*14.1 ±0.9*	*684.8 ±56.1*

TABLE V.1: Fatty acid composition of plasma total phospholipids isolated (using TLC) at specific times after burn injury was determined using GC analysis and is expressed as percent of total fatty acids. Total plasma PL was calculated using 17:0 (10 μg) as the standard and is expressed as μg/ml of plasma. Data are expressed as means ± SEM ($n \leq 10$). Significant differences between recovery timepoints were determined using a repeated measures ANOVA and values within a column not sharing common superscripts are significantly different. * indicates significant ($p<0.05$) difference from healthy subjects (reference; $n=6$).

Table V.2

Fatty acid	t1	t2	t3	t4	t5
	(µg/ml plasma)				
C 18:2(6)	79.2 ± 4.0	82.8 ± 10.5	85.2 ± 12.2	84.9 ± 18.5	98.2 ± 14.3
C 18:3(6)	0.3 ± 0.1	0.7 ± 0.2	0.5 ± 0.2	0.4 ± 0.2	0.6 ± 0.3
C 20:2(6)	1.2 ± 0.3^{a}	2.5 ± 0.4^{b}	1.2 ± 0.3^{a}	2.0 ± 0.6^{ab}	2.2 ± 0.2^{ab}
C 20:3(6)	14.6 ± 3.0	21.0 ± 4.2	25.0 ± 3.9	20.9 ± 6.7	15.7 ± 3.4
C 20:4(6)	31.0 ± 3.8^{a}	35.8 ± 4.5^{ab}	46.8 ± 5.9^{b}	38.4 ± 6.7^{ab}	46.1 ± 6.0^{b}
C 22:4(6)	0.9 ± 0.2^{a}	1.1 ± 0.1^{ab}	1.5 ± 0.3^{ab}	1.4 ± 0.5^{ab}	1.3 ± 0.2^{b}
C 22:5(6)	0.8 ± 0.1	0.8 ± 0.1	1.1 ± 0.3	1.0 ± 0.4	1.1 ± 0.4
ΣN-6	122 ± 14	144 ± 17	169 ± 22	147 ± 25	165 ± 22
C 18:3(3)	0.7 ± 0.2^{a}	0.5 ± 0.2^{a}	0.5 ± 0.2^{a}	0.9 ± 0.4^{ab}	2.0 ± 0.8^{b}
C 18:4(3)	0.9 ± 0.2	0.9 ± 0.3	0.9 ± 0.3	0.7 ± 0.3	0.7 ± 0.2
C 20:5(3)	2.2 ± 0.3	2.3 ± 0.6	1.9 ± 0.6	1.6 ± 0.2	2.0 ± 0.8
C 22:5(3)	2.9 ± 0.5	2.7 ± 0.4	3.1 ± 0.6	2.5 ± 0.4	3.3 ± 0.6
C 22:6(3)	10.0 ± 0.9^{ab}	10.2 ± 1.2^{a}	11.5 ± 2.3^{ab}	14.4 ± 4.1^{b}	12.8 ± 2.5^{ac}
ΣN-3	15 ± 2^{a}	18 ± 2^{ab}	22 ± 3^{abc}	24 ± 3^{bc}	25 ± 3^{c}
ΣPUFA	142± 15	164 ± 18	190 ± 24	172 ± 27	191 ± 24
ΣMUFA	79 ± 8	81 ± 9	86 ± 12	81 ± 14	81 ± 12
ΣSFA	212 ± 19^{a}	225 ± 23^{ab}	295 ± 30^{b}	262 ± 34^{ab}	281 ± 30^{ab}

TABLE V.2: Fatty acid composition of plasma total phospholipids expressed as µg/ml plasma calculated using 17:0 (10 µg) as the standard. Data are expressed as means ± SEM (n ≤10). Significant differences between recovery timepoints were determined using a repeated measures ANOVA and values within a row not sharing common superscripts are significantly different ($p<0.05$).

Table V.3

Post Burn Time	*PC*		*PE*		*PI*	
	Σn-6	*Σn-3*	*Σn-6*	*Σn-3*	*Σn-6*	*Σn-3*
	(% of total fatty acids)		*(% of total fatty acids)*		*(% of total fatty acids)*	
t1	31.3 ± 1.7^{ab}	3.1 ± 0.4*	31.4 ± 1.7	11.1 ± 1.0*	31.1 ± 1.2^{a*}	5.6 ± 0.6^{a}
t2	30.2 ± 1.8^{ab}	3.8 ± 0.4*	33.9 ± 1.8	11.8 ± 1.1*	35.8 ± 1.1^{b}	3.3 ± 0.6^{b}
t3	27.0 ± 1.9^{a}	2.9 ± 0.5*	33.5 ± 2.1	9.9 ± 1.2*	35.3 ± 1.4^{b}	3.9 ± 0.7^{ab}
t4	34.5 ± 2.7^{b}	2.3 ± 0.6*	31.5 ± 2.6	10.5 ± 1.5*	38.9 ± 2.1^{b}	3.2 ± 1.1^{ab}
t5	35.4 ± 2.1^{b}	3.3 ± 0.5*	34.8 ± 2.3	9.1 ± 1.3*	34.9 ± 1.6^{ab}	5.2 ± 0.9^{ab}
Reference	*31.4 ± 5.3*	*6.8 ± 1.2*	*35.7 ± 2.5*	*15.9 ± 1.5*	*35.6 ± 1.8*	*4.8 ± 0.9*

TABLE V.3 : Fatty acid composition of the PC, PE and PI fractions of plasma phospholipids expressed as percent of total fatty acids. Data are expressed as means ± SEM ($n \leq 10$). Significant differences between recovery timepoints were determined using a repeated measures ANOVA and values within a column not sharing common superscripts are significantly different. * indicates difference from healthy subjects (reference; n=6).

Table V.4

Post Burn Time	20:4n-6	Σn-6	Σn-3	ΣSFA	ΣMUFA	Total (µg/ml)
	(% of total fatty acids)					
t1	4.5 ± 0.4^{a}	$45.4 \pm 1.9^{a*}$	1.3 ± 0.1^{a}	$24.8 \pm 1.3^{a*}$	$28.1 \pm 1.2^{*}$	$336.4 \pm 28.5^{a*}$
t2	4.8 ± 0.4^{a}	$48.1 \pm 2.0^{ab*}$	1.3 ± 0.1^{a}	$24.1 \pm 1.4^{a*}$	$27.0 \pm 1.3^{*}$	$393.5 \pm 30.5^{ab*}$
t3	5.4 ± 0.5^{ab}	$47.5 \pm 2.6^{ab*}$	1.2 ± 0.1^{a}	$22.8 \pm 1.9^{ab*}$	$27.5 \pm 1.7^{*}$	$443.1 \pm 39.6^{b*}$
t4	6.0 ± 0.7^{ab}	51.4 ± 3.3^{ab}	1.1 ± 0.1^{a}	$21.6 \pm 2.3^{ab*}$	24.8 ± 2.1	$414.8 \pm 49.9^{ab*}$
t5	6.7 ± 0.6^{b}	53.0 ± 2.9^{b}	1.7 ± 0.1^{b}	18.6 ± 2.1^{b}	25.9 ± 1.9	$495.6 \pm 44.3^{b*}$
Reference	*5.3 ±0.5*	*57.6 ±2.0*	*1.2 ±0.1*	*16.6 ±1.7*	*22.0 ±1.4*	*818.5 ±40.9*

TABLE V.4 : Fatty acid composition of plasma cholesteryl esters expressed a percent of total fatty acids. Total cholesteryl esters were calculated using 15:0 (20 µg) as the standard and is expressed as µg/ml of plasma. Data are expressed as means ± SEM ($n \leq 10$). Significant differences between recovery timepoints were determined using a repeated measures ANOVA and values within a column not sharing common superscripts are significantly different. * indicates significant ($p<0.05$) difference from healthy subjects (reference; n=6).

Table V.5

Fatty acid	t1	t2	t3	t4	t5
			(µg/ml plasma)		
C 18:2(6)	138.2 ± 20.3^{a}	162.5 ± 21.7^{ab}	170.3 ± 17.2^{ab}	177.2 ± 50.2^{ab}	215.8 ± 33.3^{b}
C 18:3(6)	2.4 ± 0.6	5.4 ± 1.1	4.3 ± 0.8	3.8 ± 1.7	5.9 ± 2.4
C 20:2(6)	0.2 ± 0.1^{ab}	0.3 ± 0.1^{ab}	0.3 ± 0.0^{a}	0.0 ± 0.0^{b}	0.2 ± 0.1^{ab}
C 20:3(6)	1.7 ± 0.7	2.5 ± 0.8	2.4 ± 0.7	3.3 ± 1.6	3.6 ± 1.0
C 20:4(6)	16.3 ± 3.0^{a}	17.7 ± 3.2^{a}	23.5 ± 4.2^{ab}	24.2 ± 5.3^{ab}	33.5 ± 4.7^{b}
C 22:4(6)	0.3 ± 0.1^{a}	0.4 ± 0.2^{a}	0.7 ± 0.2^{ab}	0.2 ± 0.1^{a}	0.6 ± 0.4^{b}
C 22:5(6)	0.4 ± 0.1	0.6 ± 0.4	0.7 ± 0.4	0.8 ± 0.5	0.4 ± 0.3
ΣN-6	171 ± 20^{a}	190 ± 21^{a}	217 ± 27^{ab}	195 ± 34^{ab}	271 ± 30^{b}
C 18:3(3)	0.9 ± 0.5^{a}	0.6 ± 0.3^{a}	1.7 ± 0.7^{ab}	1.0 ± 0.6^{ab}	2.6 ± 0.8^{b}
C 18:4(3)	0.9 ± 0.1	1.0 ± 0.2	0.8 ± 0.1	0.6 ± 0.2	1.0 ± 0.1
C 20:5(3)	1.7 ± 0.4	2.1 ± 0.6	1.2 ± 0.3	0.9 ± 0.3	2.0 ± 0.4
C 22:5(3)	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.2	0.2 ± 0.2
C 22:6(3)	1.1 ± 0.3	1.7 ± 0.4	1.6 ± 0.2	0.9 ± 0.3	2.0 ± 0.3
ΣN-3	5 ± 1^{a}	5 ± 1^{a}	6 ± 1^{a}	5 ± 1^{a}	9 ± 1^{b}
ΣPUFA	165 ± 20^{a}	197 ± 19^{ab}	225 ± 25^{ab}	211 ± 31^{ab}	259 ± 28^{b}
ΣMUFA	101 ± 9	104 ± 9	113 ± 12	109 ± 15	131 ± 13
ΣSFA	71 ± 7^{a}	92 ± 7^{b}	105 ± 9^{b}	95 ± 12^{ab}	105 ± 10^{b}

TABLE V.5: Fatty acid composition of plasma CE expressed as µg/ml plasma calculated using 17:0 (10 µg) as the standard. Data are expressed as means ± SEM (n ≤10). Significant differences between recovery timepoints were determined using a repeated measures ANOVA and values within a row not sharing common superscripts are significantly different ($p<0.05$).

Table V.6

Post Burn Time	*20:4n-6*	*Σn-6*	*Σn-3*	*ΣSFA*	*ΣMUFA*	*Total (μg/ml)*
	(% of total fatty acids)					
t1	1.2 ± 0.2	15.5 ± 1.0^{a}	1.3 ± 0.1^{ab}	34.0 ± 1.0	45.8 ± 1.5	493.6 ± 53.5*
t2	1.2 ± 0.2	17.0 ± 1.1^{ab}	1.1 ± 0.2^{a}	36.1 ± 1.1*	44.9 ± 1.6	547.2 ± 58.2
t3	1.0 ± 0.3	17.3 ± 1.4^{ab}	1.3 ± 0.2^{ab}	37.1 ± 1.4	44.5 ± 2.1	515.2 ± 70.0
t4	1.4 ± 0.3	21.1 ± 1.8^{b}	1.3 ± 0.3^{ab}	35.8 ± 1.8	41.4 ± 2.7	468.2 ± 88.3*
t5	1.3 ± 0.3	19.8 ± 1.6^{b}	1.7 ± 0.2^{b}	35.0 ± 1.6	44.0 ± 2.4	481.7 ± 78.4*
Reference	*1.1 ± 0.2*	*18.2 ± 1.4*	*1.7 ± 0.3*	*31.3 ± 1.6*	*44.8 ± 1.9*	*682.7 ± 65.6*

TABLE V.6 : Fatty acid composition of plasma triglycerides expressed as percent of total fatty acids. Total triglycerides were calculated using 15:0 (20 μg) as the standard and is expressed as μg/ml of plasma. Data are expressed as means ± SEM (n ≤10). Significant differences between recovery timepoints were determined using a repeated measures ANOVA and values within a column not sharing common superscripts are significantly different ($p<0.05$). * indicates significant ($p<0.05$) difference from healthy subjects (reference; n=6).

Table V.7

V.7A.

	PC							
Post Burn Time	*18:2n-6*	*20:3n-6*	*20:4n-6*	*18:3n-3*	*20:5n-3*	*22:6n-3*	*Σn-6*	*Σn-3*
	(% of total fatty acids)			*(% of total fatty acids)*			*(% of total fatty acids)*	
t1	17.9 ± 0.7^{a}	2.1 ± 0.2^{a}	2.2 ± 0.4^{a}	0.18 ± 0.02	0.2 ± 0.1	0.5 ± 0.1	21.6 ± 1.2^{ab}	1.0 ± 0.1^{a}
t2	15.1 ± 0.7^{b}	1.4 ± 0.2^{b}	3.2 ± 0.3^{ab}	0.17 ± 0.02	0.2 ± 0.1	0.6 ± 0.1	24.2 ± 1.0^{a}	1.2 ± 1.2^{ab}
t3	17.5 ± 0.7^{a}	1.9 ± 0.2^{ab}	3.2 ± 0.3^{ab}	0.23 ± 0.02	0.2 ± 0.1	0.6 ± 0.9	23.0 ± 1.1^{ab}	1.3 ± 0.1^{ab}
t4	17.5 ± 1.0^{ab}	1.6 ± 0.3^{ab}	2.8 ± 0.5^{ab}	0.13 ± 0.03	0.1 ± 0.1	0.3 ± 0.1	19.8 ± 1.7^{b}	1.1 ± 0.2^{ab}
t5	16.2 ± 0.9^{ab}	1.5 ± 0.3^{ab}	4.0 ± 0.4^{b}	0.21 ± 0.03	0.3 ± 0.1	0.7 ± 0.1	23.9 ± 1.5^{ab}	1.6 ± 0.2^{b}
Reference[1,2]	*15.7 - 25.4*	*2.0 - 2.6*	*5.8 – 11.3*	*0 - 0.3*	*0.4 - 1.8*	*1.6 - 3.7*	*29.6 - 37.0*	*5.0 – 7.6*

V.7B.

	PE							
Post Burn Time	*18:2n-6*	*20:3n-6*	*20:4n-6*	*18:3n-3*	*20:5n-3*	*22:6n-3*	*Σn-6*	*Σn-3*
	(% of total fatty acids)			*(% of total fatty acids)*			*(% of total fatty acids)*	
t1	7.1 ± 0.3	1.0 ± 0.1^{a}	13.2 ± 1.6^{a}	0.5 ± 0.1	0.6 ± 0.1	1.8 ± 0.3^{ad}	26.8 ± 1.9^{a}	4.5 ± 0.7^{a}
t2	7.4 ± 0.4	1.2 ± 0.1^{ab}	15.4 ± 1.3^{ab}	0.5 ± 0.1	0.6 ± 0.1	2.5 ± 0.3^{ab}	28.8 ± 1.6^{ab}	5.6 ± 0.6^{ab}
t3	7.0 ± 0.3	1.5 ± 0.1^{b}	17.9 ± 1.3^{b}	0.6 ± 0.1	0.6 ± 0.1	2.7 ± 0.3^{bce}	32.7 ± 1.6^{b}	5.9 ± 0.6^{ab}
t4	8.0 ± 0.5	1.3 ± 0.2^{ab}	11.9 ± 2.0^{a}	0.4 ± 0.1	0.3 ± 0.2	1.4 ± 0.4^{d}	24.0 ± 2.5^{a}	4.2 ± 0.9^{a}
t5	7.3 ± 0.4	1.4 ± 0.2^{b}	17.8 ± 1.8^{ab}	0.5 ± 0.1	0.7 ± 0.1	3.5 ± 0.3^{e}	29.7 ± 2.2^{ab}	6.8 ± 0.8^{b}
Reference[1,2]	*6.6-10*	*1.0-2.1*	*23.8-25.8*	*0.2-0.3*	*1.6-2.9*	*7.2-12.6*	*36.8-42.4*	*14.9-20.8*

[1] Innis S (1988)

[2] Aagren (1995)

TABLE V.7 : Fatty acid composition of the PC and PE fractions of RBC expressed as percent of total fatty acids. Data are expressed as means ± SEM ($n \leq 10$). Significant differences between recovery timepoints were determined using a repeated measures ANOVA and values within a column not sharing common superscripts are significantly different ($p<0.05$).

Table V.8

Post Burn Time	PE			PC		
	ΣSFA	*ΣMUFA*	*ΣPUFA*	*ΣSFA*	*ΣMUFA*	*ΣPUFA*
t1	36.1 ± 1.0	34.0 ± 1.4^{a}	29.8 ± 2.3^{a}	53.2 ± 1.0^{ab}	24.7 ± 0.7^{a}	22.6 ± 1.2
t2	35.7 ± 1.0	31.1 ± 1.4^{ab}	33.1 ± 2.4^{ab}	51.9 ± 0.9^{ab}	22.7 ± 0.5^{b}	25.5 ± 1.1
t3	35.1 ± 1.0	29.2 ± 1.4^{b}	37.1 ± 2.4^{b}	52.7 ± 0.9^{ab}	24.4 ± 0.6^{ab}	22.8 ± 1.2
t4	36.9 ± 1.7	32.8 ± 2.3^{ab}	29.7 ± 3.8^{ab}	54.2 ± 1.4^{a}	24.5 ± 0.9^{ab}	21.4 ± 1.7
t5	34.6 ± 1.4	29.2 ± 2.0^{ab}	35.9 ± 3.3^{ab}	50.1 ± 1.2^{b}	24.0 ± 0.8^{ab}	25.9 ± 1.5

TABLE V.8: Fatty acid composition of the PC and PE fractions of RBC expressed as percent of total fatty acids. Data are expressed as means ± SEM ($n \leq 10$). Significant differences between recovery timepoints were determined using a repeated measures ANOVA and values within a column not sharing common superscripts are significantly different ($p<0.05$).

Table V.9

Post Burn Time	20:4n-6	Σn-6	Σn-3	ΣSFA	ΣMUFA	ΣPUFA
			(% of total fatty acids)			
t1	14.5 ± 1.5^{a}	25.8 ± 1.7	5.7 ± 0.8	49.8 ± 2.0	18.9 ± 1.1^{a}	32.0 ± 2.5^{a}
t2	18.6 ± 1.2^{b}	28.6 ± 1.4	7.7 ± 0.7	47.5 ± 1.7	15.6 ± 0.9^{bc}	36.9 ± 2.2^{ab}
t3	15.9 ± 1.2^{ab}	29.0 ± 1.6	7.3 ± 0.8	46.7 ± 1.9	14.8 ± 1.0^{bc}	39.8 ± 2.3^{b}
t4	15.3 ± 1.9^{ab}	25.6 ± 2.4	6.2 ± 1.2	45.3 ± 2.8	18.4 ± 1.6^{ab}	32.4 ± 3.5^{ab}
t5	20.1 ± 1.6^{b}	29.6 ± 2.1	8.1 ± 1.1	48.9 ± 2.5	13.6 ± 1.4^{c}	36.1 ± 3.1^{ab}

TABLE V.9: The fatty acid composition of the PS fraction of RBCs expressed as percent of total fatty acids. Data are expressed as means ± SEM ($n \leq 10$). Significant differences during recovery were determined using a repeated measures ANOVA and values not sharing common superscripts are significantly different ($p<0.05$).

E. References

Aarsland A, Chinkes D, Wolfe RR et al. Beta-blockade lowers peripheral lipolysis in burn patients receiving growth hormone. Rate of hepatic very low density lipoprotein triglyceride secretion remains unchanged. Ann Surg 223: 777-787, 1996.

Agren JJ, Tormala ML, Nenonen MT et al. Fatty acid composition of erythrocyte, platelet, and serum lipids in strict vegans. Lipids 30: 365-369, 1995.

Alexander JW. William A. Altemeier lecture. Nutrition and infection. New perspectives for an old problem. Arch Surg 121: 966-972, 1986.

Barton RG. Nutrition support in critical illness. Nutr Clin Pract 9: 127-139, 1994.

Batstone GF, Carlson LA, Liljedahl SO. Metabolic studies in subjects following burn injury. Burns 2: 207-211, 1976.

Birke G, Carlson LA, Liljedahl SO. Lipid metabolism and trauma. 3. Plasma lipids and lipoproteins in burns. Acta Med Scand 178: 337-350, 1965.

Blackburn GL. Nutrition and inflammatory events: highly unsaturated fatty acids (omega-3 vs omega-6) in surgical injury. Proc Soc Exp Biol Med 200: 183-188, 1992.

Blond JP, Lemarchal P., Aagaard O. A study of the effect of a-linolenic acid on the desaturation of dihomogammalinolenic acid using rat liver homegenates. Reprod Nutr Dev 24: 1-10, 1984.

Brenner R. Nutritional and hormonal factors influencing desaturation of essential fatty acids. Prog Lipid Res 20: 41-47, 1981.

Brown AJ, Pang E, Roberts DC. Erythrocyte eicosapentaenoic acid versus docosahexaenoic acid as a marker for fish and fish oil consumption. Prost Leukot Essent Fatty Acids 44: 103-106, 1991.

Caffrey BB and Jonsson HT Jr. Role of essential fatty acids in cutaneous wound healing in rats. Prog Lip Res 20: 641-647, 1981.

Calder PC. Fatty acids, dietary lipids and lymphocyte functions. Biochem Soc Trans 23: 302-309, 1995.

Calder PC. Dietary fatty acids and the immune system. Lipids 34 Suppl: S137-S140, 1999.

Carter EA and Tompkins RG. Injury-induced inhibition of fat absorption. J Burn Care Rehab 15: 154-157, 1994.

Caughey GE, Mantzioris E, Gibson RA et al. The effect on human tumor necrosis factor alpha and interleukin 1 beta production of diets enriched in n-3 fatty acids from vegetable oil or fish oil. Am J Clin Nutr 63: 116-122, 1996.

Cetinkale O and Yazici Z. Early postburn fatty acid profile in burn patients. Burns 23: 392-399, 1997.

Coombes EJ, Levick PL, Shakespeare PG. Lipid studies after burn injury in man. Burns 5: 265-268, 1979.

Coombes EJ, Shakespeare PG, Batstone GF. Lipoprotein changes after burn injury in man. J Trauma 20: 971-975, 1980.

Czaja AJ, Rizzo TA, Smith WRJ et al. Acute liver disease after cutaneous thermal injury. J Trauma 15: 887-894, 1975.

de Pablo MA and Alvarez de Cienfuegos G. Modulatory effects of dietary lipids on immune system functions. Immunol Cell Biol 78: 31-39, 2000.

Deitch EA and Sittig KM. A serial study of the erythropoietic response to thermal injury. Ann Surg 217: 293-299, 1993.

Diboune M, Ferard G, Ingenbleek Y et al. Composition of phospholipid fatty acids in red blood cell membranes of patients in intensive care units: effects of different intakes of soybean oil, medium-chain triglycerides, and black-currant seed oil. JPEN J Parenter Enteral Nutr 16: 136-141, 1992.

Fischer MA and Black HS. Modification of membrane composition, eicosanoid metabolism, and immunoresponsiveness by dietary omega-3 and omega-6 fatty acid sources, modulators of ultraviolet-carcinogenesis. Photochem Photobiol 54: 381-387, 1991.

Fukushima R, Alexander JW, Wu JZ et al. Time course of production of cytokines and prostaglandin E2 by macrophages isolated after thermal injury and bacterial translocation. Circ Shock 42: 154-162, 1994.

Gerster H. The use of n-3 PUFAs (fish oil) in enteral nutrition. Int J Vitam Nutr Res 65: 3-20, 1995.

Gottschlich MM. Selection of optimal lipid sources in enteral and parenteral nutrition. Nutr Clin Pract 7: 152-165, 1992.

Gottschlich MM and Alexander JW. Fat kinetics and recommended dietary intake in burns. JPEN J Parenter Enteral Nutr 11: 80-85, 1987.

Grbic JT, Mannick JA, Gough DB et al. The role of prostaglandin E2 in immune suppression following injury. Ann Surg 214: 253-263, 1991.

Groff JL, Gropper SS, and Hunt SM. Lipids In: Advanced Nutrition and Human Metabolism, 2, Purrington, L pp. 126-130.West Publishing Company, New York ,1995.

Harris RL, Cottam GL, Johnston JM et al. The pathogenesis of abnormal erythrocyte morphology in burns. J Trauma 21: 13-21, 1981.

Harris RL, Frenkel RA, Cottam GL et al. Lipid mobilization and metabolism after thermal trauma. J Trauma 22: 194-198, 1982.

Huang YS, Yang ZC, Liu XS et al. Serial experimental and clinical studies on the pathogenesis of multiple organ dysfunction syndrome (MODS) in severe burns. Burns 24: 706-716, 1998.

Hulsey TK, Burnham SJ, Neblett WW et al. Delayed burn wound healing in essential fatty acid deficiency. Surg For 28: 31-32, 1977.

Hulsey TK, O'Neill JA, Neblett WR et al. Experimental wound healing in essential fatty acid deficiency. J Ped Surg 15: 505-508, 1980.

Innis SM. Effect of total parenteral nutrition with linoleic acid-rich emulsions on tissue n6 and n3 fatty acids in rat. Lipids 21: 132-138, 1986.

Innis SM. The composition of red blood cell membrane phospholipids in Canadian Inuit consuming a diet high in marine mammals. Lipids 23: 1066-1067, 1988.

James MJ, Gibson RA, D'Angelo M et al. Simple relationships exist between dietary linoleate and the n-6 fatty acids of human neutrophils and plasma. Am J Clin Nutr 58: 497-500, 1993.

Kahlenberg A and Banjo B. Involvement of phospholipids in the D-glucose uptake activity of isolated human erythrocyte membranes. J Biol Chem 247: 1156-1160, 1972.

Kaufman T, Brook GJ, Hirshowitz B et al. Effect of an egg-rich diet on plasma lipids and proteins in severely burned patients. Isr J Med Sci 14: 732-740, 1978.

Kinsella JE. Lipids, membrane receptors, and enzymes: effects of dietary fatty acids. JPEN J Parenter Enteral Nutr 14: 200S-217S, 1990.

Kinsella JE, Lokesh B, Broughton S et al. Dietary polyunsaturated fatty acids and eicosanoids: potential effects on the modulation of inflammatory and immune cells: an overview. Nutr 6: 24-62, 1990.

Mittendorfer B, Jeschke MG, Wolf SE et al. Nutritional hepatic steatosis and mortality after burn injury in rats. Clin Nutr 17: 293-299, 1998.

Shen CS and Ham TH. Studies on the destruction of red blood cells. III. Mechanism and complications of hemoglobinuria in patients with thermal burns: Spherocytosis and increased osmotic fragility of red blood cells. N Engl J Med 229: 701-713, 1943.

Smith, EL, Hill, RL, Lehmann, IR et. al. Lipid Metabolism I In: Triacylglycerols in principles of biochemistry General Aspects, 7, Smith, E L, Hill, R L, Lehmann, I R et. al. pp. 508-544.McGraw-Hill Book Company, New York ,1983.

Stanford JL, King I, Kristal AR. Long-term storage of red blood cells and correlations between red cell and dietary fatty acids: results from a pilot study. Nutr Cancer 16: 183-188, 1991.

Teodorczyk-Injeyan JA, Sparkes BG, Peters WJ et al. Prostaglandin E-related impaired expression of interleukin-2 receptor in the burn patient. Adv Prost Thrombox Leukot Res 17A: 147-150, 1987.

Terry BE and Wixom RL. Hemolytic anemia associated with essential fatty acid deficiency in normal man on long term TPN. In: Fat emulsions in Parenteral nutrition, Meng, H C and Wilmore, DW. pp. 18-21.American medical association, Chicago ,1975.

Vega GL and Baxter CR. Induction of hypertriglyceridemia in rabbits by thermal injury: I. Time course of elevated plasma triglyceride levels. J Burn Care Rehab 9: 266-270, 1988.

Voss AC and Sprecher H. Regulation of metabolism of linoleic acid to arachidonic acid in rat hepatocytes. Lipids 23: 660-665, 1988.

Waymack JP, Guzman RF, Burleson DG et al. Effect of prostaglandin E in multiple experimental models. VI. Effect on T-cell subsets. Prostaglandins 38: 345-353, 1989.

Welt LG. Membrane transport defect: the sick cell. Trans Assoc Am Physicians 80: 217-226, 1967.

Wolfe RR, Herndon DN, Jahoor F et al. Effect of severe burn injury on substrate cycling by glucose and fatty acids. N Engl J Med 317: 403-408, 1987.

Wolfe RR, Shaw JH, Durkot MJ. Energy metabolism in trauma and sepsis: the role of fat. Prog Clin Biol Res 111: 89-109, 1983.

VI.

Staphyloccocus aureus Infection Reduces Natural Killer Cell Cytotoxicity and Stimulates Immune Function Following Surgery

A. Introduction

Control of wound infections in the clinical setting is critical as infections can double length of hospital stay, increase number of surgical procedures and result in delay of initiation of adjuvant therapies and reduced quality of life (Smith et al.,1994). Skin grafts are an important part of treatment for patients with open wounds. An ineffective immune system, particularly a failure of the innate component of immunity, can result in infection which affects graft success and delays wound healing (Klein et al.,1995).

Staphylococcus aureus (*S. aureus*) is a common etiological agent following surgery (Zimmerli et al.,1982). The enterotoxin produced by *S. aureus* has been classified as a superantigen. Unlike conventional antigens, superantigens bind to the external surface of the MHC II on antigen presenting cells (APCs) thereby activating a large number of T cells (Blackman et al.,1995). Early host resistance to bacterial invasions is thought to primarily depend on non T cell branches of immunity such as neutrophils and macrophages (Cantor et al.,1993). Natural killer cells, also a component of innate immunity, have been shown to lyse infected cells and directly inhibit microbial growth (Levitz et al.,1993). In addition, cytokine and chemotactic factors produced by activated T cells may also influence susceptibility to *Staphylococcal* infections (Cantor et al., 1993). B cells synthesize and secrete specific antibodies against infectious agents (Griswold,1993). Therefore, cellular components of both innate and cell-mediated immunity have important roles in defense against infections (Griswold,1993).

The impact of an impaired immune system on wound healing (reviewed in (Barbul et al.,1995) is evident in diseases like diabetes and physiological conditions such as aging. Although wound healing can proceed normally in the absence of neutrophils (Witte et al.,1997), neutrophil opsonization and killing is an absolute requirement for resistance to extracellular pathological agents (Benhaim et al.,1992). Macrophages are important phagocytes and also release growth factors and mediators necessary for wound healing. T lymphocytes release cytokines that promote wound remodelling (Barbul et al.,1989) during the final stages of wound healing.

Patients with wounds that involve substantial skin loss are at risk of both fluid loss and bacterial infection. Rapid closure of the wound aims to control these two factors, however, wound closure products and their subsequent benefits can be lost through infections caused by airborne, systemic or autochthonous microorganisms. Products that provide antimicrobial control in the clinical setting would be of great benefit. Acticoat™ is a silver-coated dressing designed for patients with substantial skin losses due to burn injury that was developed to prevent wound adhesion, limit nosocomial infection, control bacterial growth, and facilitate burn wound care (Tredget et al.,1998).

Little work has been done attempting to isolate the immunological effects of a wound as a result of surgery or injury from those of an infection. Knowing that effective immune responses are important in both wound healing and resistance to infection, the purpose of this study was to examine the effects of a surgical wound, with and without infection, on several measures of immune function that involve T and B cells, natural killer cells, macrophages and neutrophils. In addition, this study investigated the effects of a silver coated dressing (Acticoat™) on immune parameters in infected animals.

B. Methods

1. Materials

Materials utilized in this study were described in Section III.B.1. Additional experimental materials for this study were as follows: Lipopolysaccharide (E. coli O55:B5, LPS) was purchased from Sigma Chemical Co. (St. Louis, MO). Fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG and mouse anti-goat phycoerythein (PE) were purchased from Organon Teknika Inc. (Scarborough, ON). All anti-guinea pig monoclonal antibodies were purchased from Serotec (Raleigh, NC). Isoflurane was purchased from Janssen Laboratories (North York, ON) and Buprenorphine was purchased from Reckett and Coleman Pharmaceuticals Inc. Ltd. (Richmond, VA). Proviodine was purchased from Purdue Frederick Inc. (Pickering, ON). *Staphylococcus* *aureus* (strain # D05) was obtained from Alberta Provincial Laboratories (Edmonton, AB, Canada). Acticoat™ silver coated dressings were provided by the Westaim Corporation (Fort Saskatchewan, AB, Canada).

2. Animals and Surgical Procedures

All procedures were reviewed and approved by the Health Sciences Animal Welfare Committee at the University of Alberta and were consistent with Canadian Council on Animal Care guidelines.

Thirty one adult female Hartley guinea pigs (493 ± 18 g) were obtained from Charles River Canada (Laval, Quebec, Canada) and housed in the Health Sciences Laboratory Animal Services vivarium (University of Alberta, Edmonton, Alberta, Canada). Animals were acclimated in group housing in wire mesh caging and provided Purina Certified Guinea Pig Chow (St. Louis, MO), apples and water + powdered vitamin C supplement for consumption *ad libitum*. Two days prior to surgery, guinea pigs were singly housed in polycarbonate false bottom caging lined with Kimpac plastic backed absorbent material which was changed daily during the experimental period. Temperature of the rooms was maintained between 18 and 25°C, relative humidity of 50 ± 20% and a 12 h light/ dark cycle.

Following the acclimation period, animals were weighed and lightly anesthetized with isoflurane inhalant. Once anesthetized, dorsal trunk hair was clipped and depiliated with Neet™ (Carter Products Ltd, Mississauga, ON). Animals were allowed to recover from anesthesia following depiliation. On day 0 the surgical procedure was performed. All surgical procedures were performed aseptically. Animals were premedicated with buprenorphine (0.05 mg/kg subcutaneous; SQ), and anesthetized with isoflurane inhalant. The area was prepped with 70% v/v ethanol, followed by providine solution. Prior to surgery, the providine was washed off with sterile saline. The area over the dorsal midline, distal to the scapular area was insufflated with sterile saline and lubricated with sterile mineral oil. Two split thickness wounds measuring 1.5 cm x 1.5 cm x 0.33 mm were created on either side of the dorsal midline using a dermatome (Padgett Instruments Ltd. Kansas City, MO). The skin was obtained from the animal and meshed (1 to 1.5) to form an autologous skin graft. The graft was stapled in place over each wound. Wounds were maintained aseptically (Noninfected) or inoculated with *Staphylococcus aureus* at 0.5 ml of a 2×10^7 cfu/ml per wound (Infected). Wounds were dressed in either a nonadherent fine mesh dressing, or Acticoat™ silver coated dressing (Acticoat), covered by a plastic vapor barrier (sterile Saran Wrap®, Dow Chemical Ltd.) and bandaged with a 5 cm Kling halter wrap. Following surgery, animals were allowed to recover under an infrared heat lamp until they reached sternal recumbancy. Animals were returned to clean caging and recovery monitored for 24 hours. Analgesia was maintained with buprenorphine (0.05mg/kg SQ). Guinea pigs were weighed daily throughout the post-op experimental period. In order to determine the immunological response of animals not undergoing surgery, a group of guinea pigs was singly housed, handled and weighed daily but did not undergo the surgical procedure (Control). On day 5, guinea pigs were euthanized with CO_2. Blood was collected by cardiac puncture into sodium heparin Vacutainer® tubes (Becton Dickinson Ltd.) and spleens and prescalpular lymph nodes (draining the wound) were aseptically collected. Prescalpular lymph nodes (PSN) were not collected for the control animals.

3. Preparation of Lymphocytes

Splenocytes and prescalpular lymph nodes were isolated under sterile conditions by pressing through a nylon mesh (100 µm) into a petri dish and rinsing several times with Krebs- Ringer HEPES (KRH) buffer with bovine serum albumin (5 g/L). Samples were centrifuged for 10 minutes at 1000 rpm to pellet the cells, supernatants were removed and cells resuspended in warmed lysis buffer (10 ml) for 2 minutes to lyse red blood cells. KRH buffer was then added and cells were pelleted, resuspended and washed once more. Cells were resuspended in 10 ml RPMI supplemented with fetal calf serum (50 g/L), 2.5 µmol/L 2-mercaptoethanol, penicillin (100 units/mL), streptomycin (100 µg/mL) and HEPES (25 mmol/L). Cell viability was assessed using trypan blue exclusion and was greater than 99% for all groups.

4. Mitogenic Responses of Immune Cells

Splenocytes (1×10^9/L) in the media described above were cultured in 96 well microtiter plates with or without mitogens as described for the human studies (Section

IV.B.4). Mitogens used were Con A (10 mg/L), PMA (40 μg/L) plus Iono (0.5 μg/L), PHA (5 mg/L), PWM (55 mg/L) or LPS (25 mg/L). Plates were incubated for 42, 66 and 90 hours and pulsed with [^{3}H]-thymidine (18.5 kBq/well) eighteen hours prior to harvesting the cells. Both dpms and stimulation indexes were used to estimate proliferative responses to mitogens using the formula described in Section IV.B.4. Number of animals included in this analysis were noninfected, n=6; infected, n=9; Acticoat, n=6; Controls, n=10.

5. Mononuclear Cell Phenotyping

Lymphocyte subsets from spleens of the infected, noninfected and control animals as well as from the PSN (draining the wound area) for all surgery groups were characterized by immunofluorescence using supernatants from hybridomas secreting mouse mAb specific for the different guinea pig mononuclear cell subsets. MAb were preserved in 0.1% sodium azide. MAb specific for pan T cells (CD3), T helper cells (CD4), T cytotoxic cells (CD8), B cells and macrophages were used (Table VII.1). A mAb specific for MHC II was used in combination (double-labelled) with both B cells and macrophages. Fluoroscein isothiocyante (FITC; goat anti-mouse) and R-phycoerythrin (r-PE; goat anti-mouse) were reconstituted in 4% v/v guinea pig serum and used as the primary and secondary antibodies respectively. Plates were preconditioned as previously described (Section IV.B.5) and splenocytes were added to the wells (0.5x10^6 cells/well) and made up to 200 μl/well with buffer. After pelleting and vortexing the cells, the first antibodies were added to the wells and plate was incubated at 4°C for 30 minutes. The wash was repeated before adding the immunofluorescent label, FITC, after which the plate was incubated for at least 30 minutes more. Plate was washed and vortexed and r-PE was added as the secondary immunofluorescent label. Following another 30 minute incubation, cells were washed three times and fixed with 200 μl PBS + 1% (w/v) paraformaldehyde. Cells were acquired by flow cytometry on a FACScan (Becton-Dickinson, Sunnyvale, CA) and analyzed using CellQuest software. Cells incubated with FITC and r-PE alone were used to determine the amount of nonspecific binding of the imunofluourescent labels to the cells to correct for background fluorescence. For splenocyte phenotype analysis, 6 animals each from the control, infected and noninfected groups were included. For the PSN, there were 6 animals from each group including the Acticoat group. There were no PSN isolated from the nonsurgical control group.

6. Natural Killer Cell Cytotoxicity

NK cell cytotoxicity was determined on splenocytes isolated from each guinea pig using a 4 h chromium release assay as previously described for the human studies (Section IV.B.6) except that splenocytes were added in triplicate to the wells to achieve effector:target ratios of 2:1, 5:1, 10:1, 12.5:1, 25:1, 50:1, 100:1 and 200:1. Cytotoxicity against a cell line not sensitive to NK cells (P815) was measured at the 100 to 1 ratio to confirm that lysis of K562 cells was specific to NK cells only (data not shown). For this analysis, n=7 for the control and infected groups, n=9 for the non-infected group and n=6 for the Acticoat group.

7. Neutrophil Oxidative Burst

Neutrophil oxidative burst was carried out using 400 µl of whole blood deplete of red blood cells as previously described for humans (Section III.B.8) except that reaction tubes were incubated for either 5 or 15 minutes after PMA was added before they were placed on ice to stop the reaction. Non-fluorescent parameters and mean channel fluorescence of gated neutrophils was measured at 0, 5 and 15 minutes as described in Section III.B.8. For this analysis, n=6 for the non-infected and Acticoat groups, n=9 for the infected group and n=10 for the control group.

8. Statistical Analysis

Data is given as means ± SEM. The effects of treatment on [^{3}H] thymidine incorporation and phenotyping were analyzed using a one-way ANOVA and a 95% confidence interval. When a significant effect ($p<0.05$) of treatment was found, significant differences ($p<0.05$) were identified by least square means. The effect of time on mitogen responses and neutrophil oxidative burst, size and granularity was determined for each group by repeated measures ANOVA and significant differences between groups determined by least square means. NK cell activity was analyzed using a repeated measures 1-way ANOVA. All statistical analyses were conducted using the SAS statistical package (Version 6.12, SAS Institute, Cary, NC).

C. Results

1. Weight Loss

All surgery groups lost weight. There were no differences in percent weight loss after surgery between the noninfected and infected groups (noninfected = 17 ± 2%, n=6; infected = 13 ± 2%, n=9), however, the Acticoat group lost significantly more weight after surgery than the infected group (18 ± 1%, n=6).

2. Mitogenic Responses of Splenocytes

Peak response to Con A, PHA and LPS and PWM occurred at 42 hours (Table IV.1). Peak response to PMA + Iono occurred at 66 hours (Table IV.2). [^{3}H]-Thymidine incorporation into unstimulated splenocytes was significantly higher for the infected group compared to the Acticoat and control groups ($p<0.03$) at 42 h (Table VI.2A). [^{3}H]-thymidine incorporation into unstimulated cells did not differ among groups at 66 h (Table VII.2B). [^{3}H]-thymidine incorporation into unstimulated splenocytes at 90 h was significantly lower in the noninfected group compared to other groups (Table VI.2C).

At 42 and 90 h, [^{3}H]-thymidine incorporation in response to Con A was significantly lower for all groups undergoing surgery compared to the control group ($p<0.0001$; Table VI.2A, 2C). At 66 hours, only the Acticoat and infected groups had significantly lower proliferative responses to Con A than the control group (Table VI.2B). There were no differences between experimental groups in the response to PMA + Iono, PHA, and LPS and cells from all experimental groups responded lower than control cells, with the exception of the response to PMA + Iono at 90 h being higher in the infected group than the non-infected and Acticoat treated groups. For the mitogen PWM, at all timepoints, the infected group had a significantly higher rate of [^{3}H]-thymidine incorporation than the Acticoat and noninfected groups, but was not different from the control group.

3. Phenotyping

There were no significant differences in relative percentage of total T cells (pan T cells) in the spleens of the different treatment groups (Figure VI.1A). However, the control group had a significantly lower percent of both T helper (CD4+) and T suppressor (CD8+) cells compared to infected and noninfected groups ($p<0.03$). The CD4/CD8 ratio did not differ among groups (1.5 ± 0.2, data not shown). The control group had significantly lower percent of B cells ($p<0.001$) and higher percent of macrophages ($p<0.03$) than infected and noninfected groups (Figure VI.1A). Percent of B cells expressing MHC II was significantly higher in the noninfected group compared to control and infected groups ($p<0.009$; Figure VI.1B). The percentage of macrophages expressing MHC II was highest in the control group ($p<0.004$). Compared to the noninfected group the infected group had a lower expression of MHC II on both macrophages and B cells from spleen ($p<0.03$, Figure VI.1B).

Prescalpular lymph nodes from the infected pigs had significantly lower total T cells compared to the other groups but no differences in the relative percent of CD8 or CD4 cells (Table VI.3). The Acticoat group had a significantly lower CD4/CD8 ratio than the other two surgery groups due to significantly fewer numbers of CD4 cells. The Acticoat group had significantly more macrophages in their prescalpular lymph nodes. Total MHC II expression as well as expression of MHC II on both B cells and macrophages was significantly less in the Acticoat group compared to the other surgery groups (Table VI.3).

4. Natural Killer Cytotoxicity

There was no lysis of P815 cells by splenocytes demonstrating that lysis of the K562 cells was specific for guinea pig NK cells (data not shown). All surgery groups (infected, noninfected and Acticoat) had significantly lower ($p<0.001$) percent specific lysis of target cells by splenocytes at every effector:target ratio compared to the control group (Figure VI.2). The infected group had further depression of cytotoxicity ($p<0.006$) compared to the Acticoat and noninfected groups at the 100:1 ratio (Figure VI.2).

5. Neutrophil Oxidative Burst

Prior to stimulation (0 minutes), neutrophils from infected pigs had significantly greater mean channel fluorescence than the noninfected group ($p<0.001$) and the control group (Table VI.4A). After 5 minutes, neutrophils from all surgery groups had higher neutrophil responses than the control group. After 15 minutes, neutrophils from infected pigs demonstrated significantly higher mean fluorescence than the noninfected group (Table VI.4A). To determine if there were significant changes in fluorescence between 0, 5 and 15 minutes within the same group a one way ANOVA was performed between the timepoints. The percent increase in mean fluorescence was significant for neutrophils from noninfected pigs after both 5 and 15 minutes ($p<0.04$). The Acticoat group had a significant increase in mean fluorescence after 5 minutes whereas the control and infected groups did not have a significant increase in mean fluorescence from the 0 timepoint until 15 minutes ($p<0.02$; data is shown in Table II.4A but statistics are not illustrated).

Neutrophils from the Acticoat group were less granular than all other groups at all timepoints. Neutrophils from the control group were significantly more granular than the noninfected and infected groups at 15 minutes ($p<0.05$, Table VI.4B). At all timepoints, size of neutrophils from the control group was significantly less than the infected and noninfected groups ($p<0.001$; Table VI.4C). At time 0, neutrophils from noninfected pigs were significantly larger the other groups ($p<0.004$). Neutrophils from the Acticoat treated group were smaller than the infected and noninfected groups at all timepoints. Neutrophils from the control group significantly increased in size at each timepoint ($p<0.005$), neutrophils from infected pigs increased significantly from 0 to 5 minutes only ($p<0.001$) and the neutrophils from the noninfected and Acticoat groups had no significant changes in size at any timepoint (data is shown in Tables VI.4B and VI.4C but statistics are not indicated).

D. Discussion

Control of wound infection following surgical procedures remains a challenge (Holzheimer et al.,1997). It is thought that infection may result from alterations in immune function as a result of surgery (Wakefield et al.,1993). This study demonstrates that skin graft surgery (with anaesthetic) suppresses T cell proliferation and neutrophil oxidative burst. Skin graft surgery plus infection with *S. aureus* resulted in depressed natural killer cytotoxicity and MHC II expression on APCs while stimulating neutrophil oxidative burst. Treatment of an infected wound with Acticoat decreased MHC II expression on antigen presenting cells in the lymph nodes, improved NK cytotoxicity and oxidative burst in response to stimulation.

Decreased [^{3}H]-thymidine incorporation into immune cells stimulated with mitogens after surgery has been well documented (Abraham,1985; Faist et al., 1986; Slade et al., 1975; Keane et al., 1983) with reports of further reductions preceding infectious complications (Faist et al., 1986; Keane et al. 1983). In the present study there were very low responses by all surgery groups to the T cell mitogens, Con A and PHA, as well as LPS, a major B cell mitogen (Zubler et al.,1987). When cell surface structures important in cell activation were bypassed using PMA + Iono, an activator of protein kinase C and calcium ionophore respectively (Field,1995), responses remained suppressed, suggesting that the alteration in T cell function caused by surgery also involves postmembrane signals. *In vitro* response to mitogens and IL-2 secretion have been reported to be decreased by 10- to 20-fold after a *S. aureus* infection (Baschieri et al.,1993). The magnitude of reduction in mitogenic response in the present study is consistent with this report (Baschieri et al., 1993). The use of the Acticoat bandage did not improve proliferative responses of cells by infected animals.

Higher [^{3}H]-thymidine incorporation in response to PWM was observed in the infected group compared to the noninfected and Acticoat groups. PWM is mitogenic to both T and B cells but, unlike LPS, requires interactions with MHC II for mitogenesis to occur (Kasahara et al.,1979; Kina et al.,1982; Rosenberg et al.,1981). Superantigens have been reported to induce proliferation of B cells through activation involving MHC II molecules (Fullihan et al.,1991; Thibodeau et al.,1997). The lower expression of MHC II on B cells and macrophages of splenocytes from the infected group suggests that regulation of APC/T cell interactions, the target of superantigens, may be affected *in vivo*. T cells specific for the B cells that have been activated by superantigen may have been generated *in vivo* (Macy et al.,1980) and continue this response *in vitro*. Increased [^{3}H] thymidine incorporation estimates cell division and not immunoglobulin secretion or differentiation of B cells (Rosenberg et al., 1981; Kashiwa et al., 1987), therefore, B cell function was not specifically measured in this study.

There were higher proportions of lymphocytes (CD4, CD8 and B cells) in the spleens of experimental groups, however, their proliferative response to most mitogens was reduced compared to the control group further suggesting a functional impairment of these cells following surgery. Consistent with the present results, it has been reported that the CD4/CD8 ratio does not change after elective surgery (O'Mahony et al.,1984). Fewer macrophages with reduced MHC II expression observed in both noninfected and infected surgery groups may have also contributed to the reduced splenocyte proliferative response compared to the control group. Low expression of MHC II on monocytes is reported to occur after surgery and is associated with infectious complications (Wakefield et al., 1993). MHC II expression is upregulated by interferon (IFN)-γ which is produced by activated NK cells (Bancroft,1993). MHC II expression on both B cells and macrophages was reduced in the infected group which also had the lowest NK cytotoxicity.

The relative proportion of CD4 cells in the prescalpular lymph nodes was lower in the Acticoat group, compared to the other surgery groups, resulting in a reduced CD4/CD8 ratio. The Acticoat group also had greater numbers of macrophages with reduced expression of MHC II on both B cells and macrophages infiltrating the lymph nodes, compared to both the infected and noninfected groups. The implications of these findings and the impact it would have on localized immune responses are not known. Reduced MHC II expression on antigen presenting cells has been reported to occur with other types of infections (Bocca et al.,1999; Ditschkowski et al., 1999). MHC II is critical for the development and function of cells in the immune system and for activation of T cells in the periphery (Grusby et al.,1995; Pamer,1999). Therefore, these results could suggest impaired local immune responses dependent on the antigen presenting capacity of macrophages and B cells. Unfortunately splenocyte phenotypes were unavailable for this group so an overall estimate of whole body immunity is difficult.

NK cytotoxicity has been shown to be reduced following surgery (Pratt et al.,1996; Pollack et al., 1991). IL-12 is produced by macrophages (Bancroft,1993) in response to infection with *Staphylococcus* (Tripp et al.,1993; Chan et al., 1992). IL-12 increases cytolytic activity and IFN-γ synthesis by NK cells. The infected group had fewer macrophages in spleen which may have influenced both IL-12 production and IFN-γ production by NK cells. Applying the Acticoat bandage resulted in an improved NK response by the infected group.

Resistance to infection is highly dependent on neutrophil responses (Benhaim et al., 1992). There have been reports of both increased and reduced neutrophil oxidative burst following trauma (Tschaikowsky et al.,1993) and surgery (Wakefield et al., 1993). The Acticoat group had the highest oxidative response at 5 minutes that occurred with increased granularity. This suggests an improved ability of neutrophils to respond to a challenge in this group. The lower oxidative burst and granularity following stimulation suggests a failure of the noninfected group to maintain this initial response *in vitro* (Gadd et al.,1989; Utoh et al.,1988). Cytokines and circulating factors present during an infection have been shown to prime neutrophils and increase their response when stimulated (Doerfler et al.,1994; Daniels et al., 1992). The high unstimulated response suggests that neutrophils from infected pigs were primed *in vivo* to produce high amounts of oxidative compounds. Higher neutrophil oxidative burst has been reported in acute infections (Bass et al.,1986). In studies with humans, the frequency of burn wound sepsis and secondary bacteremias arising from infected burn wounds has been shown to be less frequent with the use of Acticoat compared to those treated with silver nitrate (Tredget et al., 1998). Superoxide production is an important defense mechanism against invading microorganisms in the wound area (Benhaim et al., 1992) and the observations of higher neutrophil responses by the infected and Acticoat treated groups suggests effective antibacterial function.

It has been established that the use of general anesthetics is immunosuppressive (Markovic et al.,1993b; Markovic et al., 1993a; Waymack et al., 1991). The effects of anesthetic can not be separated from those of surgery in the current study, however, general anesthetic is a necessary part of surgery, therefore, the combination of anesthetic + surgery on immune function should be viewed as being part of the immunosuppressive effects of surgery. Differences observed between the surgery groups reflect immunological effects of the infection, as anesthetic treatment was identical in all groups.

In conclusion, skin graft surgery decreased lymphocyte proliferative responses to a variety of B and T cell mitogens, reduced NK cytotoxicity and oxidative burst of neutrophils. Deficiencies in immune function caused by surgery could therefore predispose a patient to infection. Post-surgical infection further suppressed NK cytotoxicity, decreased macrophage numbers and reduced MHC II expression on antigen presenting cells which may impact on T cell/APC interactions and corresponding immune responses. Treatment of an infected wound with an Acticoat bandage improved NK cytotoxicity and increased macrophage numbers although MHC II expression on macrophages and B cells was decreased. Infection both with and without the Acticoat bandage resulted in increased oxidative burst of neutrophils which is an important defense against wound infections suggesting that stimulation of this branch of immunity may provide some early protection. The generalized failure of the cell mediated response, observed in the present study may contribute to reduced immunocompetence reported during the later stages of wound healing and some, but not all, changes may be modified by post-surgical treatment with Acticoat.

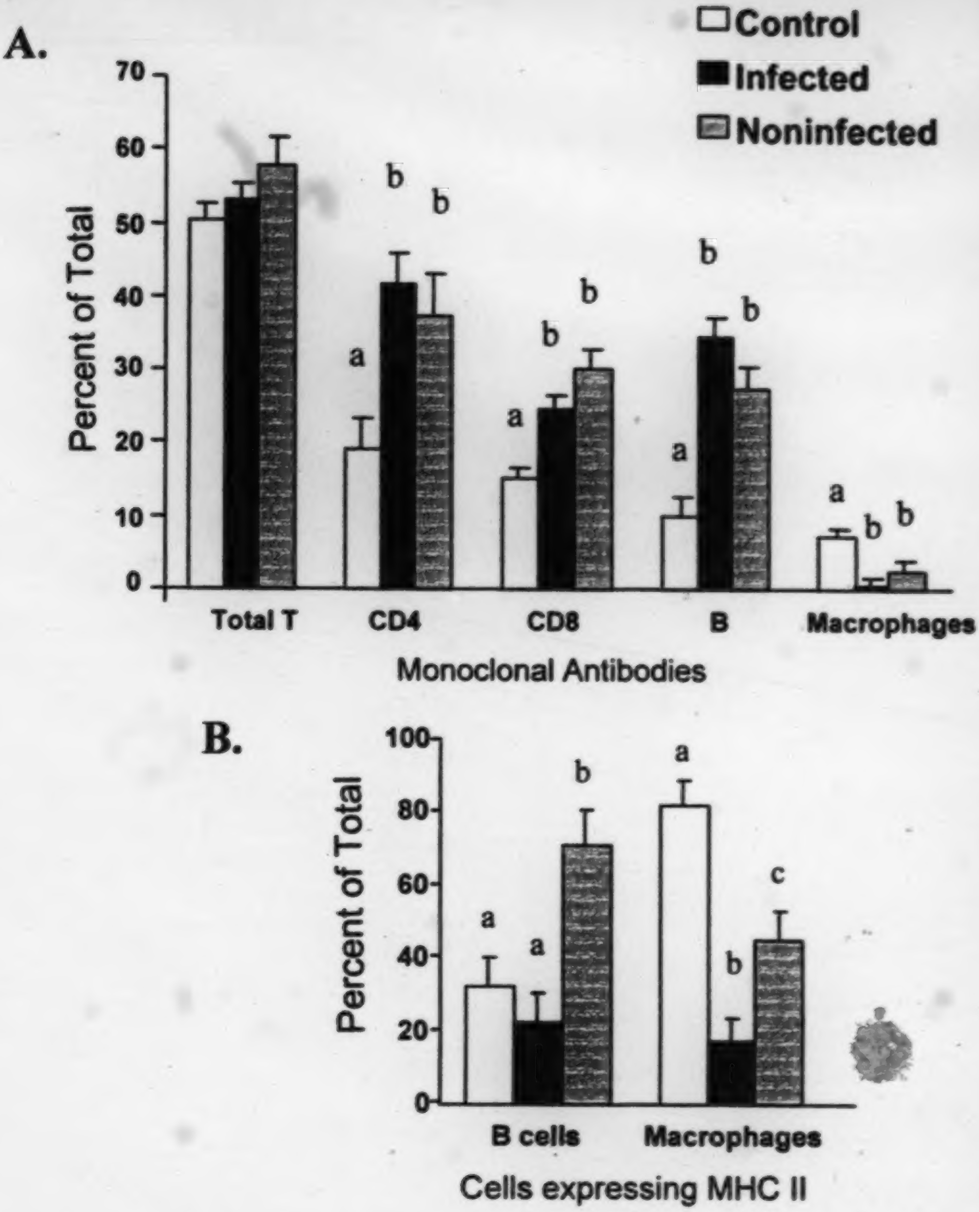


FIGURE VI.1: Effect of surgery (Noninfected), and surgery + infection (Infected) on guinea pig splenocyte phenotypes expressed as percent of total splenocytes. Bars represent mean ± SEM (n=6/group). Bars that do not share a common superscript are significantly different ($p<0.05$) as determined by a one way ANOVA and least square means.

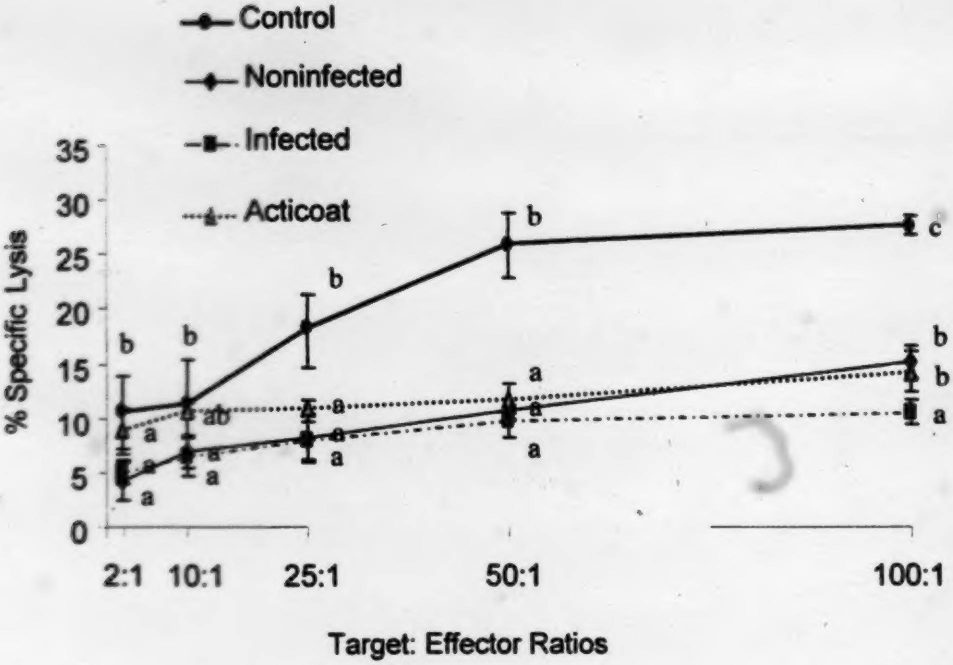


FIGURE VI.2: Effect of surgery (Noninfected), surgery + infection (Infected) and infection + Acticoat (Acticoat) on guinea pig splenocyte natural killer cell cytotoxicity determined by a $[^{51}Cr]$ release assay expressed in percent specific lysis and determined by the formula: [(experimental lysis-spontaneous lysis) / (maximum lysis -spontaneous lysis)] x 100. Points represent mean ± SEM (non-infected surgery n=10; infected surgery n=7; control n=7; Acticoat n=6) at the effector:target ratio indicated. Points that do not share a common superscript are significantly different (p<0.05) as determined by a repeated measures one way ANOVA and least square means.

Table VII.1

Monoclonal Antibody	***Isotype***	***Specific for***	***Cell Subset Identified***
MS gp12	IgG1	Guinea pig thymocytes	Total T cells
CT7	IgG1	Antigen present on a T-cell helper/inducer subset	CD4 cells
CT6	IgG1	Antigen present on T-cell suppressor/cytotoxic lymphocytes	CD8 cells
MCA518	IgG1	Cytoplasmic component of macrophages and most tissue macrophages	Macrophages
MsGp9	IgG1	Guinea pig B lymphocytes in tissue	B cells
MSgp10	IgG1	Major histocompatibility complex II on B cells and tissue macrophages	MHC II

TABLE VII.1: Specificities of the monoclonal antibodies used to identify guinea pig immune cell subsets in lymph nodes and spleen. All antibodies were purchased from Serotec (Raleigh, NC). Monoclonal antibodies were preserved in 0.1% sodium azide. Monoclonal antibodies were prepared using supernatants from hybridomas secreting mouse monoclonal antibodies specific for the different guinea pig mononuclear cell subsets.

Table VI.2

A. 42 Hours

Treatment	*US*[a]	*Con A*	*PMA+Iono*	*PHA*	*LPS*	*PWM*[b]
Noninfected	397 ± 77.4^{ab}	248 ± 152*	9587 ± 11622*	231 ± 420*	304 ± 1210*	380 ± 257^{a}*
Infected	567 ± 63.2^{a}*	473 ± 113*	38885 ± 9489*	529 ± 343*	556 ± 988*	1465 ± 210^{b}
Acticoat	265 ± 87^{b}	515 ± 273*	16557 ± 4736*	227 ± 53*	274 ± 45*	287 ± 257^{a}*
Control	335 ± 72	1290 ± 120	116309 ± 11622	2594 ± 364	4203 ± 1408	1872 ± 238

B. 66 Hours

Treatment	*US*[a]	*Con A*	*PMA+Iono*	*PHA*	*LPS*	*PWM*[b]
Noninfected	262 ± 37	359 ± 111	11099 ± 20325*	313 ± 266*	130 ± 165*	58 ± 131^{a}*
Infected	259 ± 30	308 ± 91*	52710 ± 17602*	305 ± 217*	197 ± 135*	698 ± 107^{b}
Acticoat	280 ± 96	216 ± 22*	16057 ± 1710*	276 ± 101*	198 ± 28*	227 ± 28^{a}*
Control	264± 29	648 ± 86	120424 ± 15744	1341 ± 206	838 ± 128	793 ± 101

C. 90 Hours

Treatment	*US*[a]	*Con A*	*PMA+Iono*	*PHA*	*LPS*	*PWM*[b]
Noninfected	85 ± 54^{a}*	275 ± 75*	4867± 30052^{a}*	247 ± 224*	53 ± 135*	86 ± 58^{a}*
Infected	236 ± 40^{b}	259 ± 61*	86628 ± 24538^{b}*	255 ± 183*	163 ± 110*	340 ± 47^{b}
Acticoat	225 ± 65^{b}	280 ± 59*	16316 ± 1958^{a}*	218 ± 67*	112 ± 9*	72 ± 8^{a}*
Control	243 ± 38	515 ± 58	158674 ± 23279	960 ± 173	469 ± 105	309 ± 45

[a] US=Unstimulated

[b] PWM=Pokeweed mitogen

TABLE VI.2.: Effect of surgery (Noninfected), surgery + infection (Infected) and infection + Acticoat (Acticoat) on guinea pig splenocyte response to mitogens after 42 (VI.1A), 66 (VI.1B) and 90 (VI.1C) hours measured by [^{3}H]-thymidine incorporation expressed in dpms. Values are given as mean ± SEM (noninfected n=6; infected n=9; Acticoat n=6; control n=10). Values within a mitogen grouping that do not share a common superscript are significantly different ($p<0.05$) as determined by a one way ANOVA and least mean squares. Values indicated with an * are significantly different from the control group as determined by a one way ANOVA and least square means.

Table VI.3

Immune Cell Subset	*Noninfected*	*Infected*	*Acticoat*
		(relative percent)	
Total T	87 ± 4^{b}	72 ± 4^{a}	88 ± 1^{b}
CD4	67 ± 5^{a}	60 ± 5^{a}	39 ± 4^{b}
CD8	28 ± 2	25 ± 2	31 ± 4
CD4/CD8	2.2 ± 0.2^{a}	2.4 ± 0.2^{a}	1.4 ± 0.1^{b}
B cells	30 ± 6	30 ± 6	36 ± 6
Macrophages	17 ± 2^{a}	20 ± 2^{a}	32 ± 6^{b}
MHC II[a]	12 ± 3^{b}	32 ± 4^{a}	5 ± 2^{b}
%B / MHC II	40 ± 12^{a}	37 ± 12^{a}	12 ± 6^{b}
%MO[b] / MHC II	24 ± 6^{a}	29 ± 7^{a}	2 ± 1^{b}

[a] MHC II = Major histocompatibility complex
[b] MO = Macrophages

TABLE VI.3: Effect of surgery (Noninfected), surgery + infection (Infected) and infection + Acticoat (Acticoat) on guinea pig lymphocyte subsets from the prescalpular lymph nodes. MHC II was used alone and in combination with the monoclonal antibody specific for B cells and macrophages and data is shown as percent macrophages and B cells expressing MHC II. Values are given as mean ± SEM (noninfected n=6; infected n=9; acticoat n=6). Values within a subset grouping that do not share a common superscript are significantly different ($p<0.05$) as determined by a one way ANOVA and least square means.

Table VI.4

A. Oxidative Burst (Mean Channel Fluorescence)

Treatment	*0 minutes*	*5 minutes*	*15 minutes*
Noninfected	5 ± 3^{a}	$82 \pm 7^{a*}$	172 ± 47^{a}
Infected	$22 \pm 3^{b*}$	$59 \pm 5^{b*}$	326 ± 39^{b}
Acticoat	13 ± 3^{ab}	$199 \pm 50^{c*}$	233 ± 27^{ab}
Control	6 ± 3	30 ± 7	211 ± 47

B. Granularity (Mean Side Scatter)

Treatment	*0 minutes*	*5 minutes*	*15 minutes*
Noninfected	486 ± 13^{a}	466 ± 9^{a}	$468 \pm 9^{a*}$
Infected	492 ± 11^{a}	499 ± 7^{b}	482 ± 7^{a}
Acticoat	$274 \pm 39^{b*}$	$332 \pm 9^{c*}$	$353 \pm 16^{b*}$
Control	485 ± 13	493 ± 9	506 ± 9

C. Neutrophil Size (Mean Forward Scatter)

Treatment	*0 minutes*	*5 minutes*	*15 minutes*
Noninfected	$586 \pm 14^{a*}$	$578 \pm 10^{a*}$	$595 \pm 9^{a*}$
Infected	$523 \pm 12^{b*}$	$564 \pm 9^{a*}$	$593 \pm 8^{a*}$
Acticoat	383 ± 21^{c}	386 ± 21^{b}	$399 \pm 16^{b*}$
Control	368 ± 14	403 ± 10	462 ± 9

TABLE VI.4: Effect of surgery (Noninfected), surgery + infection (Infected) and infection + Acticoat (Acticoat) on guinea pig neutrophil oxidative burst (3A), granularity (3B) and size (3C) prior to, 5 and 15 minutes after stimulation with PMA. Values are given as mean ± SEM (noninfected n=6; infected n=9; acticoat n=6; control n=10). Values within a timepoint grouping that do not share a common superscript are significantly different ($p<0.05$) as determined by a one way ANOVA and least square means.

E. References

Abraham E. Immunologic mechanisms underlying sepsis in the critically ill surgical patient. Surg Clin North Am 65: 991-1003, 1985.

Bancroft GJ. The role of natural killer cells in innate resistance to infection. Curr Opin Immunol 5: 503-510, 1993.

Barbul A, Breslin RJ, Woodyard JP et al. The effect of in vivo T helper and T suppressor lymphocyte depletion on wound healing. Ann Surg 209: 479-483, 1989.

Barbul A and Regan MC. Immune involvement in wound healing. Otolaryngol Clin North Am 28: 955-968, 1995.

Baschieri S, Lees RK, Lussow AR et al. Clonal anergy to staphylococcal enterotoxin B in vivo: selective effects on T cell subsets and lymphokines. Eur J Immunol 23: 2661-2666, 1993.

Bass DA, Olbrantz P, Szejda P et al. Subpopulations of neutrophils with increased oxidative product formation in blood of patients with infection. J Immunol 136: 860-866, 1986.

Benhaim P and Hunt TK. Natural resistance to infection: leukocyte functions. J Burn Care Rehab 13: 287-292, 1992.

Blackman MA and Woodland DL. In vivo effects of superantigens. Life Sci 57: 1717-1735, 1995.

Bocca AL, Silva MF, Silva CL et al. Macrophage expression of class II major histocompatibility complex gene products in Paracoccidioides brasiliensis-infected mice. Am J Trop Med Hyg 61: 280-287, 1999.

Cantor H, Crump AL, Raman VK et al. Immunoregulatory effects of superantigens: interactions of staphylococcal enterotoxins with host MHC and non-MHC products. Immunol Rev 131: 27-42, 1993.

Chan SH, Kobayashi M, Santoli D et al. Mechanisms of IFN-gamma induction by natural killer cell stimulatory factor (NKSF/IL-12). Role of transcription and mRNA stability in the synergistic interaction between NKSF and IL-2. J Immunol 148: 92-98, 1992.

Daniels RH, Finnen MJ, Hill ME et al. Recombinant human monocyte IL-8 primes NADPH-oxidase and phospholipase A2 activation in human neutrophils. Immunol 75: 157-163, 1992.

Ditschkowski M, Kreuzfelder E, Rebmann V et al. HLA-DR expression and soluble HLA-DR levels in septic patients after trauma. Ann Surg 229: 246-254, 1999.

Doerfler ME, Weiss J, Clark JD et al. Bacterial lipopolysaccharide primes human neutrophils for enhanced release of arachidonic acid and causes phosphorylation of an 85-kD cytosolic phospholipase A2. J Clin Invest 93: 1583-1591, 1994.

Faist E, Kupper TS, Baker CC et al. Depression of cellular immunity after major injury. Its association with posttraumatic complications and its reversal with immunomodulation. Arch Surg 121: 1000-1005, 1986.

Field CJ. A diet producing a low diabetes incidence modifies immune abnormalities in diabetes-prone BB rats. J Nutr 125: 2595-2603, 1995.

Fullihan R, Mourad W, Geha RS et al. Engagement of MHC-class II molecules by staphylococcal exotoxins delivers a comitogenic signal to human B cells. J Immunol 146: 1661-1666, 1991.

Gadd MA and Hansbrough JF. The effect of thermal injury on murine neutrophil oxidative metabolism. J Burn Care Rehab 10: 125-130, 1989.

Griswold JA. White blood cell response to burn injury. Semin Nephrol 13: 409-415, 1993.

Grusby MJ and Glimcher LH. Immune responses in MHC class II-deficient mice. Ann Rev Immunol 13: 417-435, 1995.

Holzheimer RG, Haupt W, Thiede A et al. The challenge of postoperative infections: does the surgeon make a difference?. Infect Control Hosp Epidem 18: 449-456, 1997.

Kasahara T, Kin K, Itoh Y et al. Cellular cooperation in lymphocyte activation. I. Cooperative and noncooperative responses of human T and B lymphocytes to various mitogens. Int Arch Allergy Appl Immunol 58: 260-273, 1979.

Kashiwa H, Wright SC, Bonavida B. Regulation of B cell maturation and differentiation. I. Suppression of pokeweed mitogen-induced B cell differentiation by tumor necrosis factor (TNF). J Immunol 138: 1383-1390, 1987.

Keane RM, Birmingham W, Shatney CM et al. Prediction of sepsis in the multitraumatic patient by assays of lymphocyte responsiveness. Surg Gynecol Obstet 156: 163-167, 1983.

Kina T, Nishikawa S, Katsura Y. T-cell regulation of pokeweed-mitogen-induced polyclonal immunoglobulin production in mice. I. Characterization of helper T cells. Immunol 46: 575-581, 1982.

Klein DG, Fritsch DE, Amin SG. Wound infection following trauma and burn injuries. Crit Care Nurs Clin North Am 7: 627-642, 1995.

Levitz SM and Dupont MP. Phenotypic and functional characterization of human lymphocytes activated by interleukin-2 to directly inhibit growth of Cryptococcus neoformans in vitro. J Clin Invest 91: 1490-1498, 1993.

Macy E and Stevens RH. A restricted component of T cell help in pokeweed mitogen-stimulated humans peripheral blood cell cultures. J Immunol 124: 752-759, 1980.

Markovic SN, Knight PR, Murasko DM. Inhibition of interferon stimulation of natural killer cell activity in mice anesthetized with halothane or isoflurane. Anesthes 78: 700-706, 1993b.

Markovic SN and Murasko DM. Anesthesia inhibits interferon-induced natural killer cell cytotoxicity via induction of CD8+ suppressor cells. Cell Immunol 151: 474-480, 1993a.

O'Mahony JB, Palder SB, Wood JJ et al. Depression of cellular immunity after multiple trauma in the absence of sepsis. J Trauma 24: 869-875, 1984.

Pamer EG. Antigen presentation in the immune response to infectious diseases. Clin Infect Dis 28: 714-716, 1999.

Pollack R, Lotzova E, Stanford S. Mechanism of surgical stress impairment on human perioperative natural killer cell cytotoxicity. Arch Surg 126: 338-342, 1991.

Pratt VC, Tappenden KA, McBurney MI et al. Short-chain fatty acid-supplemented total parenteral nutrition improves nonspecific immunity after intestinal resection in rats. JPEN J Parenter Enteral Nutr 20: 264-271, 1996.

Rosenberg SA and Lipsky PE. The role of monocytes in pokeweed mitogen-stimulated human B cell activation: separate requirements for intact monocytes and a soluble monocyte factor. J Immunol 126: 1341-1345, 1981.

Slade MS, Simmons RL, Yunis E et al. Immunodepression after major surgery in normal patients. Surg 78: 363-372, 1975.

Smith DJJ, Thomson PD, Garner WL et al. Burn wounds: infection and healing. Am J Surg 167: 46S-48S, 1994.

Thibodeau J, Dohlsten M, Cloutier I et al. Molecular characterization and role in T cell activation of staphylococcal enterotoxin A binding to the HLA-DR alpha-chain. J Immunol 158: 3698-3704, 1997.

Tredget EE, Shankowsky HA, Groeneveld A et al. A matched-pair, randomized study evaluating the efficacy and safety of Acticoat silver-coated dressing for the treatment of burn wounds. J Burn Care Rehab 19: 531-537, 1998.

Tripp CS, Wolf SF, Unanue ER. Interleukin 12 and tumor necrosis factor alpha are costimulators of interferon gamma production by natural killer cells in severe combined immunodeficiency mice with listeriosis, and interleukin 10 is a physiologic antagonist. Proc Natl Acad Sci USA 90: 3725-3729, 1993.

Tschaikowsky K, Sittl R, Braun GG et al. Increased fMet-Leu-Phe receptor expression and altered superoxide production of neutrophil granulocytes in septic and posttraumatic patients. Clin Investig 72: 18-25, 1993.

Utoh J, Yamamoto T, Utsunomiya T et al. Effect of surgery on neutrophil functions, superoxide and leukotriene production. Br J Surg 75: 682-685, 1988.

Wakefield CH, Carey PD, Foulds S et al. Polymorphonuclear leukocyte activation. An early marker of the postsurgical sepsis response. Arch Surg 128: 390-395, 1993.

Waymack JP, Fernandes G, Cappelli PJ et al. Alterations in host defense associated with anesthesia and blood transfusions. II. Effect on response to endotoxin. Arch Surg 126: 59-62, 1991.

Witte MB and Barbul A. General principles of wound healing. Surg Clin North Am 77: 509-528, 1997.

Zimmerli W, Waldvogel FA, Vaudaux P et al. Pathogenesis of foreign body infection: description and characteristics of an animal model. J Infect Dis 146: 487-497, 1982.

Zubler RH, Werner-Favre C, Wen L et al. Theoretical and practical aspects of B-cell activation: murine and human systems. Immunol Rev 99: 281-299, 1987.

VII.

High Dose Chemotherapy with Stem Cell Transplant Changes Neutrophil Fatty Acid Composition and Immune Function

A. Introduction

There is a poor remission prognosis beyond five years after chemotherapy treatment for metastatic breast cancer (Crown et al.,1993). Higher doses of chemotherapy have been proposed to completely destroy the tumour cells (Crown et al., 1993). Ten to 20% of the patients treated with high dose chemotherapy (HDCT) are reported to achieve long term disease free survival (Crown et al.,1995; Vahdat et al.,1997). However, the dose limiting factor in provision of this form of chemotherapy is the lethal effects it has on other rapidly proliferating cells, such as those of the immune system and gastrointestinal tract (Conners,1975). In order for patients to survive a higher dose of chemotherapy, autologous stem cell transplantation accompanies the procedure (Crown et al., 1993). Autologous stem cell transplant involves harvesting stem cells from the blood or bone marrow of the patient prior to chemotherapy (Steward,1995) and transplanting them into the patient following the treatment. It has been reported that the recovery of platelets and leukocytes is faster when blood rather than bone marrow is used for cell harvest and transplant (Ho et al.,1994; Zimmerman et al.,1995).

The treatment protocols currently being tested for patients with high risk stage II and III breast cancer and metastatic breast cancer (stage IV) at the Cross Cancer Institute (Edmonton, Alberta) include high-dose chemotherapy with autologous stem cell transplant (reviewed by McCauley,1996). Briefly, patients undergo three to six cycles of intravenously administered induction chemotherapy on an outpatient basis. Peripheral blood stem cells are harvested on day 11 or 12 after the last induction chemotherapy cycle. Approximately 2 weeks later, patients are admitted as an inpatient for the administration of intensive, high-dose chemotherapy after which they are transplanted with autologous stem cells harvested earlier (Figure VII.1). Reconstitution is defined to be clinically complete when white blood cell counts return to at least 2 x 10^9/L and platelet counts to at least 50 x 10^9/L (Ho et al., 1994). Once reconstitution is achieved, patients are discharged from the hospital.

During the period of time from administration of HDCT to reconstitution and for some time afterwards, oral food intake is limited. Intensive nutritional intervention is not currently part of the treatment protocol and patients are provided standard hospital diets. However, due to mouth soreness, gastrointestinal disturbances and taste aversions, oral food intake may be limited while in the hospital and for some time afterwards. This has not been directly studied. The effects of this treatment on essential fatty acid metabolism have not been explored.

The effects of nutritional depletion, both from preexisting cancer and the chemotherapy protocols, on the immune system have been previously reported (DeWys et al.,1980; Gross et al.,1980). Cytotoxic drugs are immunosuppressive (Harris et al.,1976) as a result of cumulative effects on a variety of cell types. These factors increase the risk for infection post-chemotherapy. Despite it being well established that restoring nutritional status has beneficial effects on patient outcome after other types of major surgery (Braga et al.,1996; Cerra et al.,1991; Daly et al.,1992), no specific nutritional intervention has been defined for women undergoing this treatment. TPN administration has been the practice in the past, however, its use was terminated when efficacy of its use was not demonstrated (Mackey,1997). Studies to date on this form of therapy have used immune cell number but not function to define immune recovery. The objective of this study was to characterize the function of the immune cells that progress at the time of transplant throughout the acute recovery period, as well as to characterize changes in the fatty acid compositions of plasma and neutrophil PL that may occur as a result of this treatment.

B. Materials and Methods

1. Subjects

The protocol for this pilot study was reviewed and approved by the Alberta Cancer Board Research Ethics Board. Six subjects were recruited from patients admitted to the Cross Cancer Institute (Edmonton, Alberta, Canada) who fit the inclusion criteria and had consented to the high dose chemotherapy procedure. Inclusion criteria included adult women residing in Edmonton or surrounding areas with poor prognosis stage II and III breast cancer (determined by physician) who were undergoing high dose chemotherapy consisting of either cyclophosphamide (6 grams/m^2), mitoxantrone (64 mg/m^2) and vinorelbine (95 mg/m^2) or cyclophosphamide, mitoxantrone and vinblastine (0.3 mg/kg) over 4 days were eligible for entry into the study. Other entrance criteria included absence of immunosuppressive or autoimmune disease, or other types of cancer, and a body mass index between 21-27. Patients requiring radiotherapy and/or surgery following the high dose chemotherapy treatment were not included in the study. Standard treatment protocols, use of antibiotics and other procedures were followed.

2. Sample Collection

Blood (16 ml) was collected into heparinized tubes by the medical staff during the patient's regular blood work at the following times (Figure VII.1):

1. At stem cell harvest (approximately 2 weeks pre-HDCT)
2. Day of discharge (approximately 2 weeks post-HDCT)

Blood was drawn from a central line by the nursing staff at the Cross Cancer Institute during the patient's regular blood work.

3. Identification of immune cell types

Identification of immune cell phenotypes was performed using whole blood as described previously in Section IV.B.5 with the following monoclonal antibodies used alone or in combinations: CD3, CD4, CD8, CD25, CD45RA, CD45RO and CD71. Monoclonal antibodies were previously described in Table IV.1.

4. Neutrophil function

After lysis of red blood cells, whole blood was incubated with DHR and stimulated with PMA for 5, 10, or 15 minutes to activate the neutrophils as previously described in Section III.B.8. Non-fluorescent (FSC and SSC) and fluorescent (FL-1) parameters were used to measure neutrophil functions as described in Section III.B.8.

5. Neutrophil Phospholipid Analysis

Lipids were extracted from Ficoll gradient isolated neutrophils and the major PL isolated as previously described in Section III.B.7. Fatty acid methyl esters were separated using gas liquid chromatography as described in Section III.B.7. with the following changes in the gas chromatography method: Injection volume was 2.5 µl, split ratio was 5:1 and run time was 50 minutes.

6. Plasma Lipid Analysis

Triglycerides (TG), cholesteryl esters (CE) and phospholipids (PL) were isolated from plasma (200 µl) as described in Section V.B.2 using the following standards to quantify plasma components: TG (10 µg 15:0), CE (20 µg 15:0) and PL (5 µg 17:0). Fatty acid methyl esters from each plasma fraction were separated using gas liquid chromatography as described in Section V.B.2 with the following changes to the method: split ratio=20:1, injection volume=2.5 µl and run time=50 minutes.

7. Statistical analysis

Differences in immune parameters, lipid composition of neutrophil PL and plasma components between time of harvest and discharge was determined using a paired T-test and level of significance was set at $p<0.05$. Statistical analysis was carried out using the SAS statistical package (Version 6.12).

C. Results

1. Subjects

Three subjects completed the study. Reasons for drop outs were as follows: two women did not reside in Edmonton or surrounding areas and would not be available for follow up and 1 began additional adjuvant treatment following the treatment. Immunological parameters at time of harvest and discharge were obtained from each the remaining three women. Harvest represents the time at which stem cells were harvested from the women (approximately 2 weeks prior to HDCT) and discharge represents the day women were discharged from the hospital (Figure VII.1). Due to failure to obtain adequate amounts of cells to perform all planned immune assays, two (lymphocyte phenotyping and neutrophil oxidative burst) were chosen based on the number of cells the assays require and the information that could be gained regarding immune cell function.

2. Cell Counts

The average time it took for reconstitution to occur (2 x 10^9/L and platelet counts to at least 50 x 10^9/L) in these subjects was 16 ± 2 days post stem cell transplant. The average number of neutrophils obtained was 1.5 ± 0.8 x 10^6/ml and 3.4 ± 1.9 x 10^6/ml at harvest and discharge respectively. Normal concentration of neutrophils defined by a complete blood count procedure is 1.8 - 7.5 x 10^9/ml and the level we previously reported for burn patients at immune recovery was 2.4 ± 0.4 x 10^9/ml (Section III.C.2). By discharge, neutrophil blood concentrations were at levels within the normal range.

3. Immune Cell Phenotypes

There were no significant differences in the proportion of $CD4^+$ and $CD8^+$ cells in peripheral blood, however, the CD4/CD8 ratio was lower ($p<0.06$) at discharge compared to harvest (Figure VII.2). All subjects had higher total expression of CD45RO (antigen mature T cells) due to a significant increase in the proportion of $CD4^+CD45RO^+$ cells (Table VII.1) at discharge compared to harvest. There were no significant differences in CD45RA (antigen naïve T cells), CD25 (IL-2 receptor) nor CD71 (transferrin receptor) expression between harvest and discharge (Table VII.1).

4. Neutrophil Functional Parameters

All subjects had a greater unstimulated and stimulated neutrophil response (increase in the mean channel fluorescence) at discharge compared to harvest (Figure VII.3A and 3B) however, the oxidative burst ratio did not differ (data not illustrated). There were no significant differences in granularity of neutrophils between harvest and discharge (data not shown). All subjects had a significant increase in the 15 minute size ratio at discharge compared to harvest (Figure VII.4).

5. Neutrophil Phospholipid Analysis

The PC fraction of neutrophil PL was proportionately higher in SFA at discharge than at harvest ($p<0.0001$; Table VII.2). The PS fraction of neutrophils contained higher amounts of MUFA, PUFA, n-6 and n-3 fatty acids and lower SFA and 20:4n-6 at discharge compared to harvest ($p<0.02$; Table VII.2, Table VII.3). The PI fraction had a three-fold reduction ($p<0.03$) in 20:4n-6 content at discharge compared to harvest (Table VII.3). There were no significant changes in the fatty acid composition of the PE fraction from time of harvest to discharge (data not shown).

The 20:4n-6 content of all PL fractions, with the exception of PC, were lower at both harvest and discharge compared to reference values (>50 d post burn injury). Other notable differences (not shown in Tables VII.2 and VII.3) were the lower PUFA levels in the PE and PI fractions at harvest and discharge (PE mean = 34.2 ± 3.3%, PI mean = 19.0 ± 1.7%) compared to reference values (PE = 44 ± 2%; PI = 29 ± 3%). A complete fatty acid profile of neutrophils isolated at harvest and discharge is given in Appendix VII.AP1.

6. Plasma PL analysis

There were no significant differences between harvest and discharge in the fatty acid composition of plasma, neither on a percent nor quantitative basis. Concentrations of plasma CE and PL were less than concentrations observed in healthy control subjects and the 20:4n-6 content of these plasma fractions tended to lower in healthy control subjects compared to the HDCT patients. The relative percents of major subsets of fatty acids and total plasma TG, CE and PL are shown in Table VII.4. A complete fatty acid profile of plasma at harvest and discharge is given in Appendix VII.AP2.

D. Discussion

Few investigations of immunological status following high dose chemotherapy with peripheral blood progenitor cell transplantation in women with breast cancer have been carried out. No data regarding essential fatty acid metabolism with this, or similar types of procedures, has been reported in the literature. Therefore, the findings from this preliminary study will be discussed in comparison to similar measures made on burn patients at 50 d post-injury when immune recovery has occurred and when available, to healthy subjects.

An important finding in this pilot study was the potential reduction in the CD4/CD8 ratio at discharge. This finding appears to be a combination of reduced CD4+ and increased CD8+ cells following the high dose procedure. Scheid *et al.* (Scheid et al.,1995) reported depressed CD4/CD8 ratios even six months after transplant. Other studies have reported increases in CD4 cells at 10 days (Ho et al., 1994) and 3 weeks post high dose chemotherapy (Scambia et al.,1993). A possible decrease in the CD4/CD8 ratio of peripheral blood following the high dose treatment might be placing patients at risk of infections as this ratio used as a clinical marker for cellular immunity (Gogos et al.,1998). Circulating T-cells have been shown to have reduced antibody and delayed type-hypersensitivity responses (Berd et al.,1982) following successive cycles of chemotherapy. Immunosuppression following other types of chemotherapy has been related to increased suppressor cell activity (Berd et al., 1982). More detailed studies are required to document possible immunological consequences of cytotoxic drugs in humans with breast cancer (Sewell et al.,1993).

CD45RO expression was higher on CD4+ lymphocytes at discharge compared to harvest and levels observed on the lymphocytes of late recovery burn patients. CD45RO expression is indicative of antigen priming suggesting lymphocytes maintain their ability to respond to antigen by antigen presenting cells. CD45RO expression is associated with capacity to proliferate and produce and respond to cytokines (Johannisson et al.,1995), suggesting the potential for improved lymphocyte function following the high dose chemotherapy treatment. Conversely, even though it has been reported that the majority of CD4+ cells emerging after bone marrow transplant are of the CD45RO isotype, their function is reported to be impaired (Clement et al.,1990). Unfortunately, the inability to obtain sufficient numbers of cells from these patients prevented investigations into the ability of lymphocytes to proliferate and produce cytokines.

Even though neutrophil number is one of the criterion for discharge, to our knowledge, the function of neutrophils following high dose chemotherapy with stem cell transplant has not been investigated. Neutrophils produce oxidative compounds that are toxic to tumor cells (Sagar et al.,1995), and they also play a pivotal role in infection defense(Dallegri et al.,1992). The change in size and production of oxidative radicals translates as improved neutrophil function from time of harvest to discharge, to levels similar to other patient groups. Lower granularity, smaller size and reduced oxidative burst responses at harvest compared to other patient groups studied, support observations of suppressed immunity as a result of chemotherapy protocols similar to those used during the induction period in this study (Berd et al., 1982; Harris et al., 1976; Sewell et al., 1993).

The effect of HDCT on essential fatty acid metabolism in PL of immune cells has not been previously investigated. This preliminary study suggests metabolism of 20:4n-6 in neutrophil PL is altered by both induction and high dose chemotherapy regimens. In all neutrophil fractions, with the exception of PC, the 20:4n-6 content was lower than reference values at both harvest and discharge. The 20:4n-6 content of the PI fraction was further reduced to a third of the harvest value by the time of discharge. Despite this, the total n-6 fatty acid content in these fractions were higher than or similar to reference values and plasma levels of 20:4n-6 appear to be similar to healthy controls. Cytotoxic drugs are known to affect phospholipase A_2 activity and arachidonic metabolism (Reviewed by (Ara et al.,1996). Neutrophils are highly dependent on cellular membranes and the increased oxidative burst observed at discharge may relate to the fatty acid content of cell membranes. Low levels of 20:4n-6 were also observed in neutrophils early after burn injury (data presented in Section III) and were associated with higher stimulated oxidative burst ratios (but not absolute production of oxidative radicals) in these patients.

The fatty acid composition of plasma components did not change with the high dose chemotherapy treatment nor were the compositions markedly different from that observed in healthy control subjects. However, the concentrations of CE and PL in plasma were less than what was observed in healthy individuals which could suggest reduced availability of fatty acids to tissues. Each plasma fraction had a trend toward lower n-3 fatty acid content prior to the HDCT treatment which may suggest increased utilization in the tissues for cellular regeneration and repair during the induction chemotherapy treatment (Caffrey et al.,1981; Dowsett,1996). Disturbances in the gastrointestinal tract and poor food intake resulting from chemotherapy may affect the availability, absorption and metabolism of fatty acids (Conners,1975). Liver toxicity has also been reported with chemotherapeutic regimens (van Berge et al.,1980; White et al.,1979) that may impair packaging of lipids into lipoproteins.

Overall, this pilot study demonstrated a marked reduction in the CD4 to CD8 ratio approximately 2 weeks after the transplant procedure. The proportion of CD4+CD45RO+ was significantly higher at discharge. The functional consequences that may result from the lower CD4/CD8 ratio may have been compensated for by the improved function of neutrophils at discharge which are an important first line of defense against infection. The essential fatty acid content of neutrophil PL was reduced following the high dose procedure, as were the concentrations of CE and PL in plasma compared to healthy controls. A high prevalence of nutrition-related problems have been reported one year following bone marrow transplantation (Lenssen et al.,1990) and the implications for acute and long term nutritional support in this group of patients warrants further investigation. Further work is needed to characterize essential fatty acid metabolism and immune function, particularly T cell function following high dose chemotherapy regimens. By doing so, immunological measures that may be improved by dietary intervention can be investigated.

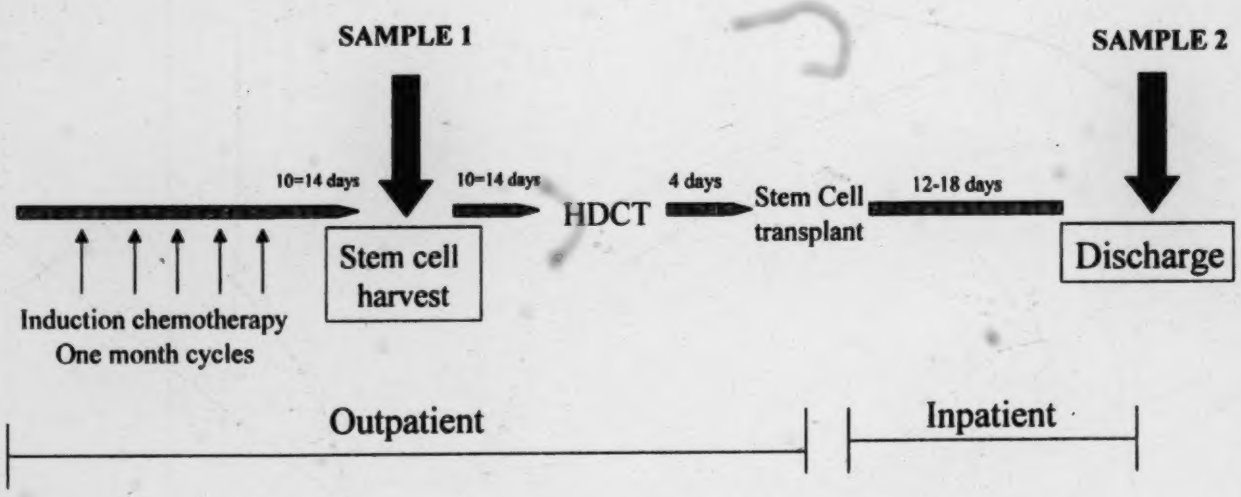

SAMPLE 1
SAMPLE 2
10=14 days
10=14 days
HDCT
4 days
Stem Cell transplant
12-18 days
Stem cell harvest
Discharge
Induction chemotherapy
One month cycles
Outpatient
Inpatient

FIGURE VII.1: A schematic of the high dose chemotherapy procedure and sampling times.

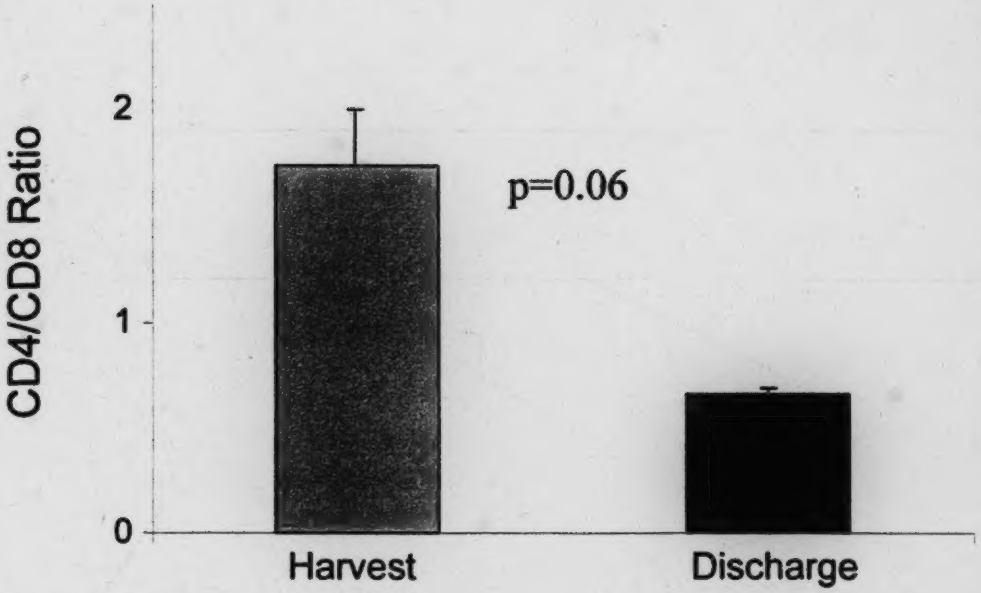


FIGURE VII.2: The CD4/CD8 ratio at harvest and discharge of women undergoing high dose chemotherapy. CD4 and CD8 cells were characterized by immunofluorescent monoclonal antibodies and analyzed using flow cytometry. Bars represent mean ± SEM (n=3). Significant differences ($p<0.05$) between timepoints was determined using a paired T-test. The dashed lines represent mean ± SEM of subjects at >50 days post burn.

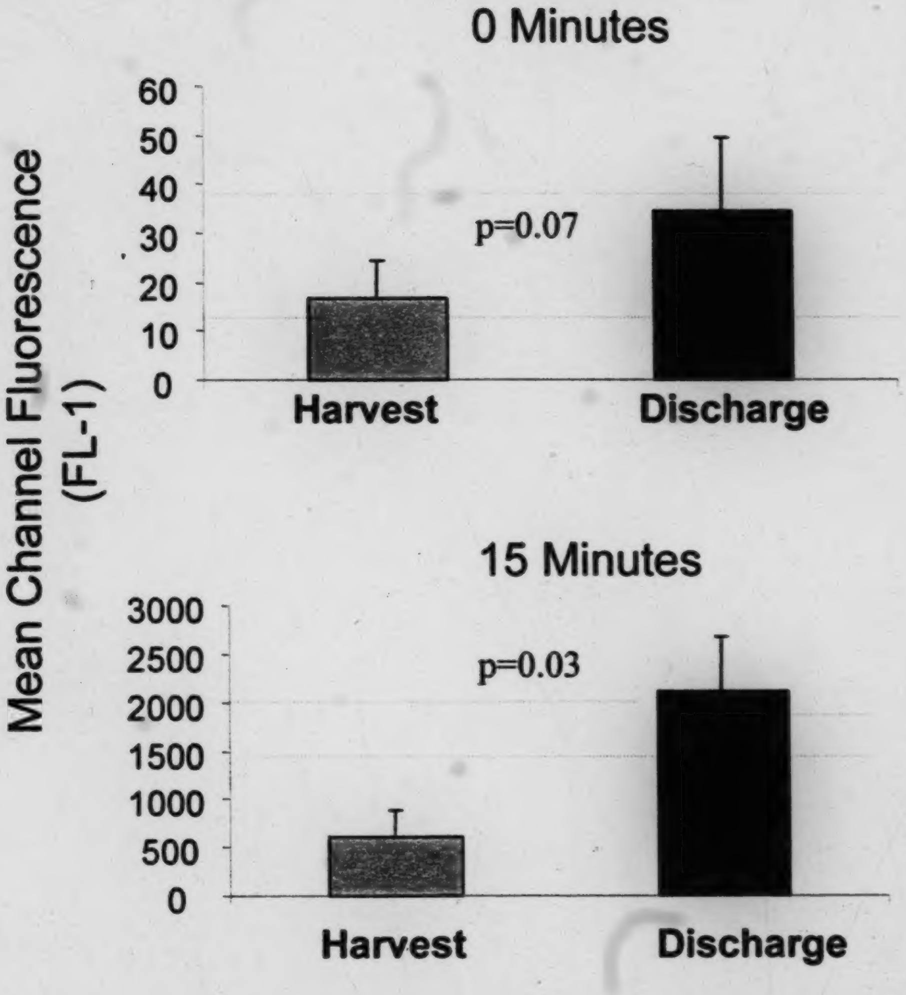


FIGURE VII.3 Effect of high dose chemotherapy on the oxidative burst of neutrophils at harvest and discharge. Mean channel fluorescence of gated cells was determined using flow cytometry to measure reduction of the DHR substrate by oxidative compounds released by the activated neutrophils before (A) and after 15 minutes (B) of stimulation with PMA *in vitro*. Bars represent mean ± SEM (n=3). Significant differences ($p<0.05$) between timepoints was determined using a paired t-test. The dashed lines represent mean ± SEM of burn subjects at >50 days post burn.

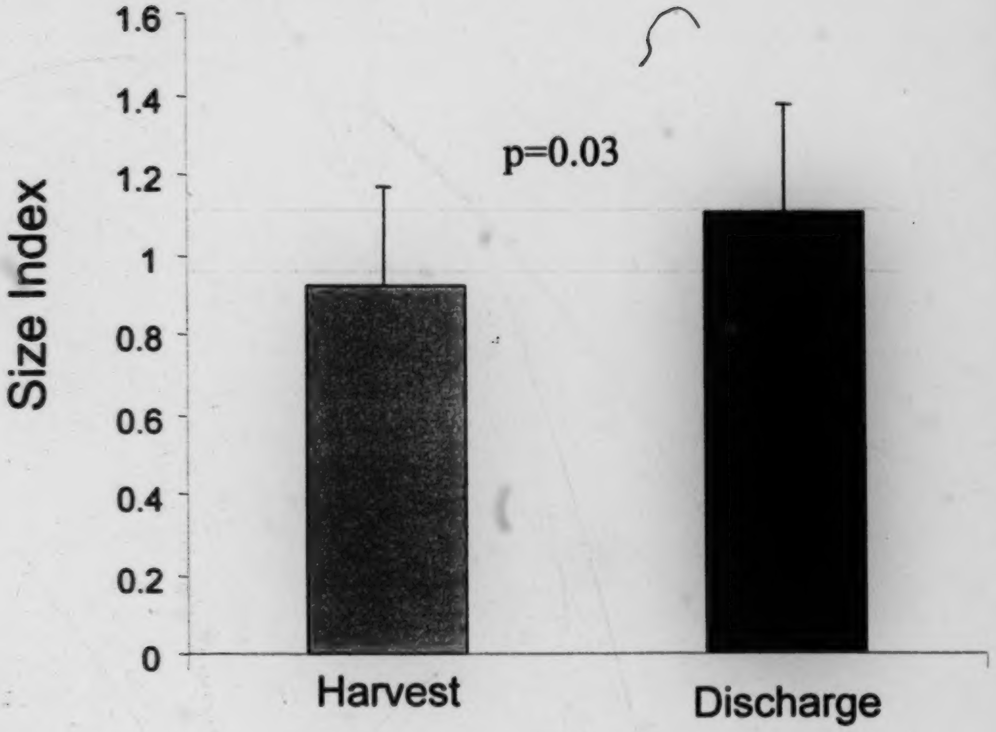


FIGURE VII.4: Effect of high dose chemotherapy on the size index of neutrophils after 15 minutes stimulation with PMA *in vitro*. Size of gated cells was determined using flow cytometry and the size ratio calculated using the formula: size at 15 minutes / size at 0 minutes. Bars represent mean ± SEM (n=3). Significant differences ($p<0.05$) between timepoints were determined using a paired t-test. The dashed lines represent mean ± SEM of burn subjects at >50 days post burn.

Table VII.1

Lymphocyte subset	Harvest	Discharge	Reference
	(relative %)		*(relative %)*
CD4	49 ± 16	40 ± 2	47 ± 5
CD8	36 ± 18	54 ± 9	35 ± 3
CD45RO	31 ±11^{a}	60 ±9^{b}	27 ± 3
CD45RA	40 ±18	22 ± 4	21 ± 6
CD25	11 ± 5	12 ± 5	22 ± 4
CD71	4 ± 4	23 ± 7	5 ± 5
CD4/CD45RA	39 ± 17	26 ± 15	42 ± 5
CD4/CD45RO	33 ± 9^{a}	59 ± 5^{b}	25 ± 7
CD8/CD45RA	46 ± 14	19 ± 0.1	32 ± 7
CD8/CD45RO	32 ± 13	48 ± 16	37 ± 10
CD4/CD25	7 ± 3	13 ± 4	20 ± 6
CD8/CD25	17 ± 5	25 ± 18	11 ± 4

TABLE VII.1: Lymphocyte subsets at harvest and discharge in women undergoing high dose chemotherapy. Lymphocyte subsets determined by using pre-labelled immunofluorescent antibodies specific for cell surface markers and analyzed using flow cytometry. Data are expressed as mean ± SEM (n=3). Differences between sampling points were determined using a paired t-test and values within a row not sharing common superscripts are significantly ($p<0.05$) different. Final reference column represents values of burn subjects at >50 d post burn but were not statistically compared to the subjects in this study.

Table VII.2

Fatty Acids	PC Harvest	PC Discharge	PC Reference	PS Harvest	PS Discharge	PS Reference
		(% fatty acids)			(% fatty acids)	
ΣSFA	46.7 ± 0.1^a	50.4 ± 0.8^b	48.1 ± 2.0	32.9 ± 0.4^a	58.4 ± 0.4^b	32.3 ± 3.1
ΣMUFA	31.7 ± 0.8	29.0 ± 0.7	27.8 ± 1.3	25.6 ± 0.6^a	21.2 ± 0.7^b	47.9 ± 3.3
ΣPUFA	21.6 ± 0.7	20.6 ± 0.6	22.3 ± 1.1	41.7 ± 0.9^a	20.4 ± 1.0^b	21.2 ± 2.4
ΣN-6	20.8 ± 1.0	20.3 ± 0.9	13.9 ± 1.9	35.9 ± 0.6^a	19.9 ± 0.6^b	16.8 ± 0.9
ΣN-3	0.9 ± 0.3	0.2 ± 0.3	3.0 ± 0.4	5.8 ± 0.4^a	0.5 ± 0.4^b	4.2 ± 1.8

TABLE VII.2: Fatty acids in the PC and PS fractions (separated by thin layer chromatography) of neutrophils isolated at harvest and discharge was determined by gas chromatography analysis and is expressed a percent of total fatty acids. Data are expressed as means ± SEM (n=3). Significant differences during recovery were determined using a paired T-test and values not sharing common superscripts are significantly ($p<0.05$) different. Reference column represents values of burn subjects at >50 d post burn but were not statistically compared to the subjects in this study.

Table VII.3

PL Fraction	*Neutrophil 20:4n-6*		
	Harvest	Discharge	Reference
	(% fatty acids)		
PC	4.3 ± 0.4	4.4 ± 0.9	6.6 ± 0.1
PE	6.5 ± 0.9	6.4 ± 0.9	19.0 ± 2.3
PS	6.7 ± 0.4^{a}	3.6 ± 0.04^{b}	9.6 ± 0.8
PI	12.4 ± 0.9^{a}	3.0 ± 1.4^{b}	16.4 ± 2.1

TABLE VII.3: Arachidonic acid content of major PL fractions (separated by thin layer chromatography) of neutrophils isolated at harvest and discharge from women undergoing high dose chemotherapy was determined by gas chromatography analysis and is expressed a percent of total fatty acids. Data are expressed as means ± SEM (n=3). Significant differences during recovery were determined using a paired T-test and values not sharing common superscripts are significantly ($p<0.05$) different. Reference column represents values of burn subjects at >50 d post burn but were not statistically compared to the subjects in this study.

Table VII.4

		Harvest	*Discharge*	*Reference*
Plasma TG				
ΣSFA		26.0 ± 0.9	23.9 ± 2.0	31.3 ± 1.3
ΣMUFA	*(% of total fatty acids)*	52.6 ± 1.0	50.1 ± 2.2	44.8 ± 1.9
ΣPUFA		21.3 ± 0.2	25.9 ± 3.3	19.9 ± 1.8
ΣN-6		18.5 ± 0.3	20.9 ± 1.6	18.2 ± 1.4
20:4n-6		2.1 ± 0.6	1.6 ± 0.3	1.1 ± 0.2
ΣN-3		2.8 ± 0.3	5.0 ± 1.5	1.7 ± 0.3
TOTAL	*(μg/ml)*	713 ± 113	672 ± 235	683 ± 66
Plasma CE				
ΣSFA		18.7 ± 5.1	12.6 ± 1.1	16.6 ± 1.7
ΣMUFA	*(% of total fatty acids)*	32.1 ± 0.4	27.9 ± 2.4	22.0 ± 1.4
ΣPUFA		49.2 ± 5.4	59.5 ± 3.3	58.8 ± 1.0
ΣN-6		48.9 ± 5.3	58.4 ± 3.5	57.6 ± 2.0
20:4n-6		9.8 ± 2.9	6.9 ± 0.74	5.3 ± 0.8
ΣN-3		0.3 ± 0.3	1.1 ± 0.2	1.2 ± 0.1
TOTAL	*(μg/ml)*	340 ± 59	475 ± 154	819 ± 41
Plasma PL				
ΣSFA		43.4 ± 0.9	41.0 ± 0.8	45.6 ± 1
ΣMUFA	*(% of total fatty acids)*	16.7 ± 1.2	16.4 ± 1.3	14.1 ± 0.9
ΣPUFA		39.9 ± 1.9	42.6 ± 2.0	39.4 ± 0.7
ΣN-6		39.8 ± 1.9	41.2 ± 3.1	35.6 ± 1.2
20:4n-6		17.2 ± 3.0	13.2 ± 1.5	9.1 ± 0.6
ΣN-3		0.1 ± 0.1	1.5 ± 1.2	3.8 ± 0.3
TOTAL	*(μg/ml)*	278 ± 50	447 ± 121	685 ± 56

TABLE VII.4: Fatty acids in plasma TG, CE and PL (separated by thin layer chromatography) at harvest and discharge from women undergoing high dose chemotherapy were determined by gas chromatography analysis and is expressed a percent of total fatty acids. Total plasma TG, CE and PL were calculated based on standards added as described in the methods. Data are expressed as means ± SEM (n=3). Significant differences during recovery were determined using a paired T-test and values not sharing common superscripts are significantly ($p<0.05$) different. Final column represents values from healthy subjects (Reference; n=6) but were not statistically compared to the subjects in this study.

E. References

Ara G and Teicher BA. Cyclooxygenase and lipoxygenase inhibitors in cancer therapy. Prost Leukot Essent Fatty Acids 54: 3-16, 1996.

Berd D, Mastangelo MJ, Engstrom PF et al. Augmentation of the human immune resposne by Cyclophosphamide. Cancer Res 42: 4862-4866, 1982.

Braga M, Vignali A, Gianotti L et al. Immune and nutritional effects of early enteral nutrition after major abdominal operations. Eur J Surg 162: 105-112, 1996.

Caffrey BB and Jonsson HTJr. Role of essential fatty acids in cutaneous wound healing in rats. Prog Lip Res 20: 641-647, 1981.

Cerra FB, Lehmann S, Konstantinides N et al. Improvement in immune function in ICU patients by enteral nutrition supplemented with arginine, RNA, and menhaden oil is independent of nitrogen balance. Nutrition 7: 193-199, 1991.

Clement LT, Vink PE, Bradley GE. Novel immunoregulatory functions of phenotypically distinct subpopulations of CD4+ cells in the human Neonate. J Immunol 145: 102-108, 1990.

Conners, TA. Mechanisms of Action of 2-chloroethylamine derivatives, sulfur mustards, epoxides and aziridines In: Antineoplastic and Immunsuppressive Agents, Part II, Sartorelli, A C and Johns, D G pp. 18-34.Springer-Verlag, New York ,1975.

Crown J and Norton L. Potential strategies for improving the results of high-dose chemotherapy in patients with metastatic breast cancer. Ann Oncol 6 Suppl 4: 21-26, 1995.

Crown J, Vahdat L, Fennelly D et al. High-intensity chemotherapy with hematopoietic support in breast cancer. Ann N Y Acad Sci 698: 378-388, 1993.

Dallegri F and Ottonello L. Neutrophil--mediated cytotoxicity against tumour cells: state of art. Arch Immunol Ther Exp (Warsz) 40: 39-42, 1992.

Daly JM, Lieberman MD, Goldfine J et al. Enteral nutrition with supplemental arginine, RNA, and omega-3 fatty acids in patients after operation: immunologic, metabolic, and clinical outcome. Surg 112: 56-67, 1992.

DeWys WD, Begg C, Lavin P et al. Prognostic effect of weight loss prior to chemotherapy in cancer patients. Am.J.Med. 69:491-497, 1980

Dowsett J. Nutrition--module 1: Part 4--The importance of nutrition in wound healing. World Ir Nurs 4: 15-18, 1996.

Gogos CA, Ginopoulos P, Salsa B et al. Dietary omega-3 polyunsaturated fatty acids plus vitamin E restore immunodeficiency and prolong survival for severely ill patients with generalized malignancy: a randomized control trial. Cancer 82: 395-402, 1998.

Gross RL and Newberne PM. Role of nutrition in immunologic function. Physiol Rev 60: 188-302, 1980.

Harris JE, Sengar D, Stewart TH et al. The effect of Immunosuppressive chemotherapy on immune function in patients with malignant disease. Cancer 37: 1058-1069, 1976.

Ho AD, Maruyama M, Maghazachi A et al. Soluble CD4, soluble CD8, soluble CD25, lymphopoieitic recovery, and endogenous cytokines after high-dose chemotherapy and blood stem cell transplantation. Blood 84: 3550-3557, 1994.

Johannisson A and Festin R. Phenotype transition of CD4+ T cells from CD45RA to CD45R0 is accompanied by cell activation and proliferation. Cytometry 19: 343-352, 1995.

Lenssen P, Sherry ME, Cheney CL et al. Prevalence of nutrition-related problems among long-term survivors of allogeneic marrow transplantation. Journal of the American Dietetic Association 90: 835-842, 1990.

Mackey J. Nutrition in high dose chemotherapy patients. 1997.

McCauley DL. High-dose chemotherapy with stem-cell rescue for the treatment of breast cancer. Am J Health-Syst Pharm 53: 521-534, 1996.

Sagar SM, Singh G, Hodson DI et al. Nitric oxide and anti-cancer therapy. Cancer Treat Rev 21: 159-181, 1995.

Scambia G, Panici PB, Pierelli L et al. Immunological reconstitution after high-dose chemotherapy and autologous blood stem cell transplantation for advanced ovarian cancer. Eur J Cancer 29A: 1518-1522, 1993.

Scheid C, Pettengell R, Ghielmini M et al. Time-course of the recovery of cellular immune function after high-dose chemotherapy and peripheral blood progenitor cell transplantation for high-grade non-Hodgkin's lymphoma. Bone Marr Transplant 15: 901-906, 1995.

Sewell HF, Halbert CF, Robins RA et al. Chemotherapy-induced differential changes in lymphocyte subsets and natural-killer-cell function in patients with advanced breast cancer. Int J Cancer 55: 735-738, 1993.

Steward WP. High dose chemotherapy with haemopoietic support for Breast cancer. Eur J Cancer 31A: S21-S24, 1995.

Vahdat L and Antman K. High-dose chemotherapy with autologous stem cell support for breast cancer. Curr Op Hematol 4: 381-389, 1997.

van Berge H and Vogten AJ. Drug-induced liver injury. Netherlands J Med 23: 23-32, 1980.

White DR, Cooper MR, Muss HB et al. Complications of cytotoxic antineoplastic chemotherapy. N Car Med J 40: 73-78, 1979.

Zimmerman TM, Mick R, Myers S et al. Source of stem cells impacts on hematopoietic recovery after high-dose chemotherapy. Bone Marrow Transplant 15: 923-927, 1995.

VIII.

Improvements in Immune Functions and Alterations in Plasma and Neutrophil PL Composition After Fish Oil Supplementation in Weight Losing Cancer Patients

A. Introduction

Cancer and therapies used to treat it result in impaired immune responses which are further exacerbated by poor nutritional status. Weight loss of 5% of is associated with immune deficits and an increased risk of mortality (DeWys et al.,1980). Cachexia is a multifactorial complication of cancer characterized by anorexia, weight loss, muscle loss, and abnormalities in macronutrient metabolism (Wigmore et al.,1997). Fat mobilization from adipose tissues (Kitada et al.,1982; Taylor et al.,1992) and oxidation of free fatty acids have been reported to be increased (Douglas et al.,1990; Hansell et al.,1986). Plasma lipid compositions of cancer patients has been reported to differ from healthy individuals or those with benign disease (Chaudry et al.,1991; McClinton et al.,1991). In patients with advanced pancreatic cancer, supplemental n-3 fatty acids in the form of fish oil have been shown to prevent further weight loss and promote weight gain in some patients (Barber et al.,1999; Wigmore et al.,1996; Wigmore et al.,1997). The mechanisms for these effects have not been entirely elucidated, however, reduced production of catabolic cytokines, reduced tumour growth, stimulation of anticancer immune defenses of the host, and an attenuation of the acute phase response have been proposed.

Lymphocytes and neutrophils play important roles in anti-cancer defense (Dallegri et al.,1992; Robins,1986). Cellular and innate immune functions have been reported to be impaired in cachexia and malnutrition (Gross et al.,1980). Immune cells are known to be affected by changes in the fatty acid composition of their cellular PL (reviewed in Section I.F). The ability of cachexic patients to absorb and metabolize n-3 fatty acids is not well-characterized. Whether these fatty acids get incorporated into host tissues or cells of the immune system is not known nor at what dose is required to see these effects. The objectives of this study were to examine appearance of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in plasma and immune cells and investigate immune parameters after 14 days of supplementation of fish oil to cancer patients who are experiencing weight loss.

B. Methods

1. Materials

The source of all materials purchased for this study are described in Sections III.B, IV.B and V.B.

2. Subjects

Patients with advanced cancer (defined as locally recurrent or metastatic) at the Acute Palliative Care Unit and Hospice, Caritas Health Group and both hospitalized and outpatients at the Cross Cancer Institute (Edmonton, Alberta, Canada) were eligible for entry into this study. Inclusion criteria included presence of anorexia (defined as more than 3 in a 0-10 visual analogue scale), weight loss of 5% or more of their pre-illness weight, the ability to maintain oral intake, normal cognition (defined as a normal Mini-Mental State examination for age and level of education) and the ability and willingness to give written, informed consent. Eligible participants were randomized using a computer generated randomized sequence to receive identical gelatin capsules containing fish oil (180mg EPA and 120 mg DHA, Banner Pharmacaps, Olds, AB, Canada) or placebo oil (olive oil). Random allocation to each treatment arm took place at the Pharmacy Department, Cross Cancer Institute. Sealed envelopes were used to allocate patients to either fish oil or placebo groups. All investigators and health care workers were blinded to the treatment received throughout the trial. Subjects were instructed to take 18 capsules each day for 14 days. On day 0 and day 14 of supplementation, a sample of peripheral blood (16 ml) was taken by a registered nurse during in-home visits or in the inpatient clinic. Twenty subjects were assigned to receive placebo oil and 25 were assigned to receive fish oil during the duration of the study.

3. Preparation of Neutrophils

Neutrophils were isolated using Ficoll Hypaque gradient centrifugation as previously described in Section III.B.6. Plasma was removed from the top of the gradient and frozen immediately at -70°C. Cell viability was assessed using trypan blue exclusion and was greater than 99% for all samples. Cells were pelleted and frozen immediately at -70°C until lipid analysis.

4. Phospholipid Fatty Acid Analysis

Previously frozen plasma from the top of the gradient was thawed and 100 µl transferred to a glass tube. Lipids were extracted from the sample using chloroform/methanol (2:1) as described in Section V.B.2. Chloroform (200 µl) was added to the dried sample and divided into 2 tubes for duplicate sampling of plasma. Phospholipids (PL) were isolated from other plasma components using "G" plates as described in Section V.B2.

A modified Folch method was used to extract lipids from isolated neutrophils as previously described in Sections III.B.7. Individual PL were separated on thin layer chromatography "H" plates as previously described in Section III.B.7. The PL band from the G-plates and PI, PS, PC and PE on the H-plates were identified and scraped into methylation tubes. Standards were added to the serum PL band (10 µg C17:0) and cellular PL bands (40 µg C17:0).

Samples were methylated using BF_3 and hexane as described in Sections III.B.7 and V.B.7. After samples cooled, 2 ml of distilled water were added and samples kept at 4°C overnight. The upper layer containing the fatty acid methyl esters was removed, placed in a GC vial and dried down under nitrogen. Fatty acid methyl esters from the plasma PL fraction were separated by an automated gas-liquid chromatograph as described in Section V.B.7. Fatty acid methyl esters from neutrophil PL were separated using a Varian CP 3800 gas liquid chromatograph with the following changes in the method described in Section III.B.7: Split ratio=5, run time=60 minutes and injection volume=5 µl. Fatty acid content of plasma PL was calculated from the internal standard added. Percents of saturated, monounsaturated, polyunsaturated, n-6 and n-3 fatty acids as well as EPA, DHA and 20:4n-6 content were analyzed in all PL fractions.

5. Neutrophil Oxidative Burst

Estimation of granularity and oxidative burst of neutrophils was carried out using 400 µl of whole blood deplete of red blood cells as previously described in Section III.B.6. Mean channel fluorescence (FL-1), mean forward (FSC, size) and side (SSC; granularity) scatters of gated neutrophils were measured at 0, 5, 10 and 15 minutes. The change in granularity and oxidative burst after stimulation was determined using a granularity or oxidative burst ratio given by the formula (5,10 or 15 minute value/0 minute value).

6. Mononuclear Cell Phenotyping

Whole blood (100 µl) was added to wells of a microtiter plate. Red blood cells were lysed using warm lysis buffer and immune cell subsets characterized by immunofluorescence assay as described previously in Section IV.B.5. Monoclonal antibodies were described in Section IV.B.5 and specificities are shown in Table IV.1. CD28 (Sigma, St. Louis, MO) was an additional antibody used and is specific for the CD80 ligand on antigen presenting cells. The three-colour combinations used to identify lymphocyte subsets are shown in Table VIII.2.

7. Statistical Analysis

Data is reported as mean ± SEM. To measure changes in immune parameters and fatty acid composition within a supplementation group, a paired T-test was used. Differences between groups and timepoints were analyzed using a repeated measures ANOVA Significant differences ($p<0.05$) between treatments and day 0 and 14 were identified using least square means. All statistical analyses were conducted using the SAS statistical package (Version 6.12, SAS Institute, Cary, NC).

C. Results

1. Subjects

Twenty-three subjects completed the study. Reasons for noncompliance included failure to maintain intake, complications due to cancer progression and death. Thirteen and 10 subjects completed 14 days of fish oil (FO) and placebo supplementation, respectively and their characteristics are shown in Table VIII.2. These groups did not differ significantly in age, body weight or the presence of metastasis. The primary site of the cancer varied widely among subjects in both groups. Number of capsules consumed by the subjects did not differ significantly between groups. The FO group consumed on average 6 to 17 capsules per day (mean=12 ± 1) and the placebo group consumed on average 6 to 18 capsules per day (mean=10 ± 1).

2. Fatty Acid Composition of Plasma PL

All subjects in the FO group exhibited increases in the EPA content of plasma PL after 14 d supplementation (Figure VIII.1). The mean percent increase in EPA and DHA from day 0 was 495 ± 39% and 125 ± 20%, respectively (Table VIII.3). Quantitatively, EPA increased from 0.7 ± 0.4 μg/ml plasma to 4.3 ± 0.4 μg/ml plasma and DHA increased from 3.1 ± 0.4 μg/ml plasma to 4.6 ± 0.4 μg/ml plasma. Fish oil supplementation resulted in a significant reduction in 18:2n-6 but no change in the 20:4n-6 content in plasma PL (Table VIII.8). Total n-6 fatty acids and the n6/n3 ratio of plasma PL were significantly reduced after 14 days of FO supplementation (Table VIII.3).

The placebo group did not exhibit significant changes in plasma PL fatty acid composition. Compared to the FO group, the percent MUFA was higher and percent PUFA lower in plasma PL after 14 days of supplementation. Both the FO and Placebo group had lower plasma PL concentrations than healthy individuals (Table VIII.3). The level of plasma PL did not increase with fatty acid supplementation in either group. The complete fatty acid profile of plasma PL is shown in Appendix VIII.AP1.

3. Fatty Acid Composition of Neutrophil PL

The PI fraction of neutrophil PL exhibited significant reductions in total PUFA and 20:4n-6 content, and an increased content of MUFA after fish oil supplementation (Table VIII.4). There were no significant differences between pre and post FO supplementation in any other major PL fractions and percent of EPA and DHA in neutrophil PL did not change with 14 days of supplementation (Table VIII.2).

4. Neutrophil Function

There were no significant alterations in neutrophil oxidative burst (Table VIII.5), size or granularity (Table VIII.6) with FO supplementation. The placebo oil group exhibited a significant increase in the basal response (0 minutes) following supplementation making the 0 minute value (unstimulated response) significantly higher in the placebo compared to the fish oil group post-supplementation ($p<0.008$; Table VIII.5). Granularity and granularity ratios of neutrophils from both groups were lower than values observed at >50 days post burn injury (Table VIII.6).

5. Lymphocyte Phenotypes

There were no changes associated with supplementation in the relative percent of CD4 or CD8 cells or the CD4/CD8 ratio in either group (Table VIII.7). The proportions CD8 cells expressing CD25 significantly increased in the FO group from 0 to 14 days (Table VIII.7). The FO group had a significant reduction in CD28 expression on CD8 cells post-supplementation. The proportion of B cells was significantly higher after 14 days of fish oil supplementation compared to the placebo group (Table VIII.7). Despite randomization, prior to supplementation the two groups differed significantly in CD4/CD8 ratio, expression of CD45RA, CD45RO and CD25 on CD8 cells (Table VIII.7).

D. Discussion

The ability of supplemental fish oil to reduce the cachexic response in patients with advanced cancer has been recently demonstrated (Barber et al., 1999; Wigmore et al., 1996; Wigmore et al., 1997). Our study supports that dietary fats from fish oil can be absorbed and incorporated into plasma lipids but there is a low incorporation of these fatty acids into neutrophil cellular membranes after 14 days of supplementation. This is important in that incorporation into membranes is a proposed mechanism for the effects of fish oil on the favourable clinical response by cachexic cancer patients (Wigmore et al., 1997). Neutrophil function following supplementation with EPA and DHA in cachexic cancer patients has not been previously investigated. Feeding cachexic cancer patients fish oil for 14 days resulted in a lower unstimulated response of neutrophils compared to subjects fed the placebo treatment. This could suggest a lower *in vivo* production of oxidative radicals. High free radical production could potentially be harmful to the host and might indicate an increased infection rate (Fujishima et al.,1995). Infection has been shown to increase the unstimulated production of oxidative radicals (Wakefield et al.,1993). Dietary fish oils have been reported to reduce the incidence of infection of other critically ill patients (Table I.F.2). There was a trend toward increased stimulated oxidative burst response in the fish oil supplemented group. The response by neutrophils in this study was within ranges observed in other patient groups (Section III.C.3) suggesting maintenance of a key effector function important in tumor toxicity (Dallegri et al., 1992) and infection defense (Gadd et al.,1989).

The proportion of B cells in the fish oil supplemented group increased after 14 days, and approached values reported for healthy individuals (Abbas et al.,1994b). Changes in the proportion of B cell numbers in cachexic cancer patients has not been reported previously. Tumor bearing hosts have been shown to produce antibodies against tumour antigens that contribute to antibody-dependent cell mediated cytotoxicity by macrophages or natural killer cells (Abbas et al.,1994a). Of greater importance in immune surveillance, however, are the CD8+ cells which are cytotoxic against tumor cells (Melief,1992). Anti-tumour activity mediated by CD8 cells is stimulated by cytokines produced by the TH1 and TC1 subset of lymphocytes, IL-2 and IFN-γ (Mosmann et al.,1997). An upregulation of the IL-2 receptor (CD25) is suggestive of activation in response to IL-2. Dietary fish oils have been shown to increase the proportions of CD8+CD25+ and the production of TH1 cytokines by splenocytes of tumor-bearing rats (Robinson,2000). To induce an 8-fold increase in CD25 expression within 14 days is a novel and exciting finding. Other studies have reported immune improvements after 40 days (Gogos et al.,1995) and 6 months (Purasiri et al.,1995) but our data suggests that changes in CD8 mediated immunity may occur by 2 weeks of supplementation. The clinical importance of reduced CD28 expression in the fish oil supplemented group requires further investigation as CD28 has been shown to aid in overcoming mechanisms used by the tumour to evade host defenses, such as preventing T-cell anergy (Allison,1994) and apoptosis (Harding et al.,1992). The observations of changes specific to CD8+ cells with fish oil supplementation which serve important roles in anti-cancer defense provides an exciting direction for further research.

The percent increase in plasma EPA and DHA after 14 days of fish oil supplementation was similar to levels reported in other studies that have used longer intervention periods (Barber et al., 1999; Wigmore et al., 1997). However, the concentration of plasma PL in all subjects in this study (pre and post-supplementation) were nearly one eighth of that observed in healthy individuals (Table VIII.3). This finding has not been previously reported. Prior to supplementation, the proportion of EPA and DHA content in plasma was similar to levels observed in healthy subjects, however, the marked reduction in plasma PL concentrations results in significantly less EPA and DHA in the plasma on a quantitative basis. Tumours or presence of metastasis in sites important in fatty acid metabolism would be expected to interfere with lipoprotein synthesis and composition (Wigmore et al., 1997), however, this finding was observed in all cancer patients regardless of tumor site. Several factors could contribute to lower plasma PL concentrations. Poor appetite and reduced dietary intake are common among terminal stage cancer patients (Feuz et al.,1994; McCann et al.,1994) and a high proportion of all cancer patients have been reported to develop some degree of clinical malnutrition (Kern,1988; Ollenschlager et al.,1991). Additionally, the elevated levels of cytokines, reported during cachexia (Tisdale,1999) impact on fatty acid metabolism (Grunfeld et al.,1991; Hardardottir et al.,1992). Alterations in fatty acid metabolism are characterized by increased fatty acid oxidation, lipolysis, and accelerated clearance of endogenous and exogenous fat sources in both the fed and fasting state (Douglas et al., 1990; Kitada et al., 1982; Mulligan et al.,1991; Mulligan et al.,1992; Waterhouse et al.,1971; Wigmore et al., 1997). Reduced dietary intake coupled with an increased metabolic rate may exert cumulative effects on plasma lipoprotein concentrations.

Other abnormalities in fatty acid metabolism have been reported in cancer patients. In patients with bladder and prostate cancer, the plasma content of 18:1 has been reported to be increased with reductions in 18:2n-6, 20:4n-6, EPA and DHA as compared to healthy controls (Chaudry et al., 1991; McClinton et al., 1991). These plasma profiles would be consistent with deficiencies in essential fatty acids (Gottschlich et al.,1987). The present study confirms low levels and proportions of 18:2n-6 in the plasma of cancer patients, however, no change in the 20:4n-6 content or composition of plasma occurred with FO supplementation.

Neutrophils isolated both before and after fish oil supplementation did not differ in the percent EPA and DHA content in cellular PL. The low concentrations of plasma PL may have limited the availability and incorporation of these fatty acids into cell membranes, despite 14 days of supplementation. Increased fatty acid oxidation rates that have been reported to occur in cachexic states may have resulted in oxidation of the supplemented fatty acids rather than their incorporation into cellular membranes. N-3 fatty acids have been reported to exert immunomodulatory effects on immune cell functions by changing membrane fluidity, enzyme activities and intracellular signals (reviewed in Section I.F), however, these fatty acids need to be incorporated into the cell before they can exert these effects. Appearance of EPA in cellular lipids has been demonstrated after only 2-6 weeks (Lee et al.,1985) of fish oil supplementation in healthy humans, with no changes in 20:4n-6 content. Although no change in EPA or DHA content in the PL factions of neutrophils was produced after 14 days of supplementation with fish oil, the 20:4n-6 content of PI was reduced by half after the supplementation period. PI is linked to a major receptor binding site that enhances neutrophil activation (Hundt, 1992) and it has also been shown to be an important intracellular signalling molecule in these cells (Tissot et al.,1991). N-3 fatty acids have been shown to inhibit 20:4n-6 metabolism and incorporation into PL (Gerster,1995; Virella et al.,1991; von Schacky et al.,1985). Surprisingly, there appeared to be no relationship between number of capsules consumed, types of cancer or other patient characteristics and the levels of DHA and EPA found in their membranes.

In conclusion, this study is the first to report low levels of plasma PL in cancer patients experiencing weight loss. EPA, rather than DHA, was more consistently increased in the plasma PL of the subjects consuming fish oil. There was a limited incorporation of either EPA or DHA content in neutrophil PL, although a significant reduction in 20:4n-6 was observed when fish oil was consumed. Of the immune cell phenotypes examined, CD8 cells appear to most affected by fish oil supplementation and there was a large increase in the expression of CD25 on these cells after 14 d of fish oil supplementation. This is suggestive of improved anti-tumour responses but requires further investigation of functional parameters. In the placebo treatment group, but not the fish oil, there was a higher unstimulated oxidative burst production by neutrophils, which may suggest *in vivo* activation of neutrophils. Our results confirm that feeding fish oil to cancer patients for a 2 week period can alter the composition and activation of peripheral immune cells. The recent reports of attenuated weight loss with dietary fish oil supplementation provides exciting evidence for the ability of complex metabolic disease processes to be altered by nutritional intervention and warrants further investigations into utilization of n-3 fatty acids by the host.

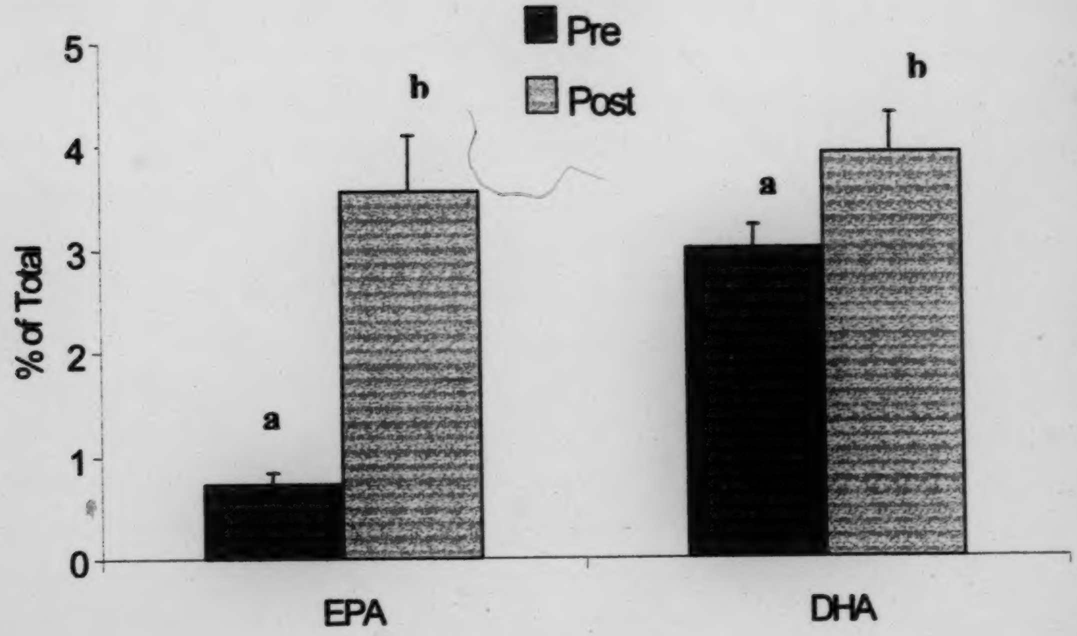


FIGURE VIII.1: Percent of EPA and DHA in plasma PL before and after 14 days of supplementation with fish oil (n=13). Plasma PL were isolated using G-plates and fatty acid composition determined using gas liquid chromatography. Significant ($p<0.05$) differences were determined using a paired t-test.

Table VIII.1

Lymphocyte Subsets			
MAb 1	*Mab 2*	*Mab 3*	*Rationale*
CD4	CD45RO	CD45RA	To determine the expression of antigen exposure and naivity phenotypes on T cell subsets
CD8	CD45RO	CD45RA	
CD4	CD28	CD25	To determine the expression of IL-2 receptor and CD28 molecule on T cell subsets
CD8	CD28	CD25	
CD3	CD28	CD25	
CD20			To determine relative percent of B cells

TABLE VIII.1: Three-colour combinations of monoclonal antibodies used to identify lymphocyte subsets.

Table VIII.2A

Patient ID	*Age (yrs)*	*Gender*	*Weight (kg)*	*Cancer site*	*Meta-stasis*	*Mean # capsules*	*EPA/d (g)*	*DHA/d (g)*
CG	48	M	73	pancreas	No	16	2.8	1.9
JT	65	M	87	prostate	Yes	17	3.1	2.1
AB	61	F	N/A	lung	No	8	1.5	1.0
IM	73	M	54	rectum	Yes	8	1.4	0.9
LH	58	M	68	rectum	yes	17	3.1	2.1
ES	67	M	92	prostate	yes	12	2.2	1.4
OB	82	M	63	jejunum	yes	11	2.1	1.4
GK	69	F	54	rectum	yes	16	2.9	1.9
BI	78	M	59	lung	no	6	1.1	0.7
PS	61	F	49	breast	yes	6	1.1	0.7
KM	77	F	62	adenocarcinoma	yes	12	2.2	1.4
SW	46	F	44	unknown	yes	9	1.6	1.1
RW	61	M	70	Cecum	yes	16	2.8	1.9
mean	*65 ± 3*		*65 ± 4*			*12±1*	*2.3 ± 0.2*	*1.5 ± 0.2*

TABLE VIII.2.A: Characteristics of subjects randomly allocated to receive fish oil.

Table VIII.2B

Patient ID	*Age (yrs)*	*Gender*	*Weight (kg)*	*Cancer site*	*Metastasis*	*Mean # capsules*
RG	69	M	73	lung	yes	10
HH	63	M	73	kidney	Yes	18
HP	77	M	45	parotid	yes	8
JL	81	M	67	prostate	Yes	14
KC	59	M	54	leiomyosarcoma	yes	6
AH	73	M	58	lung	no	9
CT	66	M	50	stomach	yes	15
PB	62	M	55	rectum	yes	6
CL	76	M	60	colon	yes	7
RO	65	M	63	lung	yes	7
mean	*69 ± 2*		*60 ± 3*			*10 ± 1*

TABLE VIII.2B: Characteristics of subjects randomly allocated to receive placebo oil.

Table VIII.3

Fatty acid	*Pre FO*	*Post FO*	*Pre Placebo*	*Post Placebo*
		(% of total fatty acids)		
C18:2 (6)	18.0 ± 0.8^{a}	15.2 ± 0.6^{b}	17.0 ± 0.9^{ab}	17.8 ± 1.1^{a}
C 20:2(6)	0.6 ± 0.1^{a}	0.3 ± 0.0^{b}	0.5 ± 0.1^{ab}	0.4 ± 0.1^{ab}
C 20:3(6)	1.2 ± 0.1	1.4 ± 0.2	1.6 ± 0.1	1.6 ± 0.3
C 20:4(6)	8.7 ± 0.4	7.9 ± 0.4	8.6 ± 0.4	7.6 ± 0.4
C 22:4(6)	0.2 ± 0.0^{a}	0.1 ± 0.1^{b}	0.3 ± 0.0^{a}	0.2 ± 0.1^{a}
C 22:5(6)	0.3 ± 0.1	0.0 ± 0.0	0.3 ± 0.1	0.3 ± 0.1
Σn6	*28.7 ± 1.6^{a}*	*25.0 ± 1.2^{b}*	*27.9 ± 1.8^{ab}*	*26.5 ± 1.6^{ab}*
C 20:5(3)	0.7 ± 0.2^{a}	3.8 ± 0.6^{b}	0.5 ± 0.1^{a}	0.5 ± 0.1^{a}
C 22:5(3)	0.6 ± 0.1^{a}	1.0 ± 0.1^{b}	0.7 ± 0.0^{c}	0.6 ± 0.0^{a}
C 22:6(3)	2.9 ± 0.3^{a}	4.1 ± 0.5^{b}	3.2 ± 0.5^{ab}	2.8 ± 0.3^{a}
C 20:5(3)	0.7 ± 0.2^{a}	3.8 ± 0.6^{b}	0.5 ± 0.1^{a}	0.5 ± 0.1^{a}
C 22:5(3)	0.6 ± 0.1^{a}	1.0 ± 0.1^{b}	0.7 ± 0.0^{c}	0.6 ± 0.0^{a}
C 22:6(3)	2.9 ± 0.3^{a}	4.1 ± 0.5^{b}	3.2 ± 0.5^{ab}	2.8 ± 0.3^{a}
N6/N3 Ratio	6.8 ± 0.6^{a}	2.9 ± 0.6^{b}	6.9 ± 0.7^{a}	6.8 ± 0.6^{a}
ΣPUFA	36.4 ± 1.2^{a}	36.2 ± 1.2^{a}	34.5 ± 2.2^{ab}	32.6 ± 2.0^{b}
ΣSFA	47.8 ± 2.3	49.5 ± 2.3	49.4 ± 2.6	50.8 ± 2.6
ΣMUFA	15.8 ± 1.0^{ab}	14.3 ± 1.0^{a}	16.0 ± 1.0^{ab}	16.5 ± 0.9^{b}
Total PL (μg/ml)	*112.3 ±47.4*	*89.9 ±47.4*	*67.9 ±50.7*	*79.1 ±44.7*

TABLE VIII.3: The fatty acid content of plasma PL expressed a percent of total fatty acids. Total plasma PL was calculated using the internal standard (10 μg C17:0) and is expressed as μg/ml. Data are expressed as means ± SEM (FO, n=13, Placebo, n=10). Significant differences between groups were determined using repeated measures ANOVA and differences identified using least square means. Values within a column not sharing common superscripts are significantly ($p<0.05$) different. Values for healthy subjects are available in Appendix V.AP4.

Table VIII.4

	SFA	MUFA	PUFA	20:4n-6	EPA	DHA
			PI			
Pre FO	45.7 ± 5.4	10.7 ± 3.1^a	48.9 ± 6.8^a	26.8 ± 4.2^a	2.1 ± 1.2	2.5 ± 0.9
Post FO	49.0 ± 4.0	20.8 ± 2.3^b	30.3 ± 5.0^b	13.7 ± 3.1^b	1.6 ± 0.9	1.4 ± 1.2
Reference	*57.4 ±3.2*	*12.9 ±1.2*	*28.1 ±4.7*	*14.9 ±3.4*	*0.7 ±0.3*	*0.8 ±0.2*
			PC			
Pre FO	39.7 ± 2.7	28.7 ± 2.3	33.6 ± 3.4	14.1 ± 2.9	0.2 ± 0.1	0.7 ± 0.4
Post FO	39.1 ± 2.2	33.6 ± 1.9	27.6 ± 2.8	11.9 ± 2.3	0.1 ± 0.1	0.8 ± 0.3
Reference	*46.1 ±1.2*	*29.6 ±0.5*	*24.2 ±0.8*	*7.8 ±0.8*	*1.9 ±1.0*	*0.3 ±0.2*
			PE			
Pre FO	27.6 ± 4.3	24.9 ± 2.9	40.3 ± 4.2	26.3 ± 3.1	0.2 ± 0.2	1.1 ± 1.0
Post FO	37.7 ± 3.9	29.0 ± 2.6	31.4 ± 3.7	23.0 ± 3.1	0.3 ± 0.2	1.1 ± 0.8
Reference	*40.3 ±5.6*	*22.2 ±1.8*	*38.6 ±4.9*	*21.4 ±4.1*	*0.4 ±0.1*	*1.9 ±0.3*
			PS			
Pre FO	39.9 ± 3.9	22.6 ± 3.4	37.5 ± 4.7	19.2 ± 3.6	0.6 ± 0.4	1.4 ± 0.8
Post FO	37.5 ± 3.2	26.1 ± 2.8	36.4 ± 3.9	16.4 ± 2.9	0.4 ± 0.4	1.2 ± 0.6
Reference	*32.1 ±2.7*	*48.3 ±3.0*	*20.8 ±1.7*	*9.6 ±0.8*	*0.2 ±0.1*	*0.9 ±0.4*

TABLE VIII.4: Fatty acid composition of major PL fractions of neutrophils before and after 14 days of fish oil supplementation (n=12) expressed as a % of fatty acids. Major PL were isolated from gradient isolated neutrophils using TLC and fatty acid composition determined using gas liquid chromatography. Significant differences ($p<0.05$) between pre and post FO supplementation was determined using a t-test. Reference values of fatty acids are shown (healthy control subjects, n=4 for PC, PE and PI, and >50 days post burn injury for PS, n=6) but were not statistically compared to the subjects in this study.

Table VIII.5

Stimulation time	*Pre-FO*	*Post-FO*	*Pre-Placebo*	*Post-Placebo*	*Reference*
	Mean channel fluorescence (FL-1)				
0 minutes	21.7 ± 7.4^a	25.0 ± 6.8^a	31.7 ± 5.9^a	57.8 ± 10.6^b	28.0 ± 5.5
5 minutes	268.9 ± 78.8	213.0 ± 72.9	413.8 ± 105.7	414.2 ± 72.2	412.1 ± 95.2
5 Minute Index	17.7 ± 3.2	11.0 ± 2.9	11.8 ± 4.2	7.3 ± 2.2	19.2 ± 6.5
10 minutes	819.0 ± 154.2	810.6 ± 142.8	1029.2 ± 208.7	1192.4 ± 124.6	1244.0 ± 165.4
10 minute Index	59.7 ± 11.0	46.6 ± 10.2	37.3 ± 13.7	32.2 ± 11.2	67.8 ± 27.7
15 minutes	1422.9 ± 191.8	1392.1 ± 177.5	1671.3 ± 243.9	1842.7 ± 175.6	1820.8 ± 196.8
15 minute Index	104.2 ± 20.6	85.8 ± 19.1	73.1 ± 24.0	45.4 ± 23.2	91.9 ± 30.5

TABLE VIII.5: Oxidative burst of neutrophils before and after 14 days of supplementation with fish oil (n=13) or placebo (n=10). Freshly isolated neutrophils were stimulated with PMA for 5, 10 and 15 minutes *in vitro* and production of oxidative radicals quantified using flow cytometry. The index at each timepoint was determined using the formula: Mean channel fluorescence (5, 10 and 15 minutes) / 0 minute Mean channel fluorescence. Data are expressed as means ± SEM. Significant differences between groups were determined using a repeated measures ANOVA and values within a row not sharing common superscripts are significantly ($p<0.05$) different. The control values are those from burn patients at >50 days post burn (Section III.C.3) but were not statistically compared to the subjects in this study.

Table VIII.6

Stimulation time	Pre-FO	Post-FO	Pre-Placebo	Post-Placebo	Reference
	Mean Granularity (SSC)				
0 minutes	521.1 ± 14.5	554.3 ± 13.9	545.4 ± 15.9	550.0 ± 16.7	742.3 ± 32.6
5 minutes	522.8 ± 11.8	533.1 ± 11.4	516.0 ± 11.6	523.6 ± 12.3	723.6 ± 33.2
5 minute Index	1.0 ± 0.02	0.96 ± 0.02	0.95 ± 0.02	0.96 ± 0.02	0.98 ± 0.03ab
10 minutes	484.9 ± 13.4	520.8 ± 12.9	498.7 ± 14.0	502.0 ± 14.8	708.2 ± 34.2
10 minute Index	0.93 ± 0.02	0.93 ± 0.02	0.92 ± 0.02	0.91 ± 0.02	1.01 ± 0.03^{b}
15 minutes	478.4 ± 13.6	478.4 ± 11.8	473.6 ± 12.9	473.1 ± 13.6	691.4 ± 38.1
15 minute Index	0.93 ± 0.02	0.90 ± 0.02	0.87 ± 0.02	0.86 ± 0.02	1.03 ± 0.02^{c}

TABLE VIII.6: Neutrophil granularity before and after 14 days of supplementation with fish oil (n=13) or placebo (n=10). Freshly isolated neutrophils were stimulated with PMA for 5, 10 and 15 minutes *in vitro* and granularity measured using mean SSC of gated cells by flow cytometry. The index at each timepoint was determined using the formula: Mean SSC (5, 10 and 15 minutes) / 0 minute SSC. Data are expressed as means ± SEM. Differences between groups were determined using a repeated measures ANOVA and values not sharing common superscripts are significantly ($p<0.05$) different. The control values are those from burn patients at >50 days post burn (Table III.6) but were not statistically compared to subjects in this study.

Table VIII.7

Lymphocyte Marker	*Pre FO*	*Post FO*	*Pre Placebo*	*Post Placebo*
	(Relative Percent)			
CD4	48.6 ± 2.9	45.5 ± 4.8	39.8 ± 3.1	35.8 ± 3.6
CD8	28.1 ± 2.4	32.9 ± 3.2	34.2 ± 2.6	35.5 ± 3.5
CD4/CD8 Ratio	1.9 ± 0.2^{a}	1.8 ± 0.2^{ab}	1.1 ± 0.2^{b}	1.2 ± 0.2^{b}
CD20	7.0 ± 0.8^{ab}	10.8 ± 1.2^{a}	6.2 ± 1.4^{ab}	5.4 ± 0.9^{b}
CD4/CD45RA	14.0 ± 3.6	8.9 ± 1.9	15.9 ± 4.0	11.9 ± 2.1
CD4/CD45RO	53.6 ± 4.9	50.9 ± 5.0	51.1 ± 5.3	58.6 ± 5.5
CD4/CD25	17.1 ± 5.5	14.5 ± 6.0	28.1 ± 6.1	22.9 ± 6.5
CD8/CD45RA	46.8 ± 6.0^{a}	60.2 ± 3.8^{ab}	69.7 ± 6.6^{b}	68.8 ± 4.2^{ab}
CD8/CD45RO	33.3 ± 3.0^{a}	28.8 ± 2.6	24.5 ± 3.3^{b}	28.5 ± 2.9
CD3/CD25	28.4 ± 4.8	30.9 ± 5.4	26.9 ± 5.2	33.0 ± 5.9
CD8/CD25	3.3 ± 4.4^{a}	23.1 ± 7.5^{b}	30.5 ± 4.8^{b}	39.1 ± 8.2^{b}
CD4/CD25	17.1 ± 5.5	14.5 ± 6.0	28.1 ± 6.1	22.9 ± 6.5
CD3/CD28	74.1 ± 3.3	62.9 ± 6.4	83.3 ± 3.6	71.9 ± 7.0
CD4/CD28	85.5 ± 5.7	83.9 ± 5.7	73.7 ± 8.1	91.8 ± 8.1
CD8/CD28	39.7 ± 7.8^{a}	23.0 ± 5.5^{b}	28.1 ± 7.8^{ab}	38.3 ± 7.8^{ab}

TABLE VIII.7: Lymphocyte subsets were determined by performing immunoflourescence on whole blood of subjects consuming fish oil (n=13) or placebo (n=10) for 14 days. Pre-labelled monoclonal antibodies specific for cell surface markers indicated were used to determine relative percents of lymphocytes bearing the specific cell surface antigen. Data are expressed as means ± SEM. Differences between groups were determined using a repeated measures ANOVA and values not sharing common superscripts are significantly ($p<0.05$) different.

E. References

Abbas AK, Lichtman AH, and Pober JS. Cells and Tissues of the immune system In: Cellular and Molecular Immunology, 2, Abbas AK, Lichtman AH, and Pober JS pp. 15-30.W.B. Saunders, Pennsylvania ,1994b.

Abbas AK, Lichtman AH, and Pober, JS. Immunity in Defense and Disease In: Cellular and Molecular Immunology, 2, Abbas AK, Pober JS, and Lichtman AH pp. 368.W.B. Saunders Company, Pennsylvania, Philidelphia ,1994a.

Allison JP. CD28/B7 interactions in T cell activation. Curr Opin Immunol 6: 414-419, 1994.

Barber MD, Ross JA, Preston T et al. Fish-oil enriched nutritional supplement attenuates progression of the acute-phase response in weight-losing patients with advanced pancreatic cancer. J Nutr 129: 1120-1125, 1999.

Chaudry A, McClinton S, Moffat LE et al. Essential fatty acid distribution in the plasma and tissue phospholipids of patients with benign and malignant prostatic disease. Br J Cancer 64: 1157-1160, 1991.

Dallegri F and Ottonello L. Neutrophil--mediated cytotoxicity against tumour cells: state of art. Arch Immunol Ther Exp (Warsz) 40: 39-42, 1992.

DeWys WD, Begg C, Lavin P et al. Prognostic effect of weight loss prior to chemotherapy in cancer patients. Am J Med 69:491-497, 1980.

Douglas, R. G. and Shaw, J. H. Metabolic effects of cancer. Br.J.Surg. 77:246-254, 1990

Feuz A and Rapin CH. An observational study of the role of pain control and food adaptation of elderly patients with terminal cancer. J Am Diet Assoc 94: 767-770, 1994.

Fujishima S and Aikawa N. Neutrophil-mediated tissue injury and its modulation. Int Care Med 21: 277-285, 1995.

Gadd MA and Hansbrough JF. The effect of thermal injury on murine neutrophil oxidative metabolism. J Burn Care Rehab 10: 125-130, 1989.

Gerster H. The use of n-3 PUFAs (fish oil) in enteral nutrition. Int J Vitam Nutr Res 65: 3-20, 1995.

Gogos CA, Ginopoulos P, Zoumbos NC et al. The effect of dietary omega-3 polyunsaturated fatty acids on T-lymphocyte subsets of patients with solid tumors. Canc Detect Prevent 19: 415-417, 1995.

Gottschlich MM and Alexander JW. Fat kinetics and recommended dietary intake in burns. JPEN J Parenter Enteral Nutr 11: 80-85, 1987.

Gross RL and Newberne PM. Role of nutrition in immunologic function. Physiol Rev 60: 188-302, 1980.

Grunfeld C, Soued M, Adi S et al. Interleukin 4 inhibits stimulation of hepatic lipogenesis by tumor necrosis factor, interleukin 1, and interleukin 6 but not by interferon-alpha. Cancer Res 51: 2803-2807, 1991.

Hansell, D. T., Davies, J. W., and Burns, HJG. The relationship between resting energy expenditure and weight loss in benign and malignant disease. Ann.Surg. 203:240-245, 1986

Hardardottir I, Doerrler W, Feingold KR et al. Cytokines stimulate lipolysis and decrease lipoprotein lipase activity in cultured fat cells by a prostaglandin independent mechanism. Biochem Biophys Res Commun 186: 237-243, 1992.

Harding FA, McArthur JG, Gross JA. CD28 mediated signalling costimulates murine T cells and prevents induction of anergy in T cell clones. Nature 356: 607-609, 1992.

Kern PA. Recombinant human tumor necrosis factor does not inhibit lipoprotein lipase in primary cultures of isolated human adipocytes. J Lipid Res 29: 909-914, 1988.

Kitada S, Hays E, Mead JF et al. Lipolysis induction in adipocytes by a protein from tumor cells. J Cell Biochem 20:409-416, 1982

Lee TH, Hoover RL, Williams JD et al. Effect of dietary enrichment with eicosapentaenoic and docosahexaenoic acids on in vitro neutrophil and monocyte leukotriene generation and neutrophil function. N Engl J Med 312: 1217-1224, 1985.

McCann RM, Hall WJ, Groth-Juncker A. Comfort care for terminally ill patients. The appropriate use of nutrition and hydration. JAMA 272: 1263-1266, 1994.

McClinton S, Moffat LE, Horrobin DF et al. Abnormalities of essential fatty acid distribution in the plasma phospholipids of patients with bladder cancer. Br J Cancer 63: 314-316, 1991.

Melief CJ. Tumor eradication by adoptive transfer of cytotoxic T lymphocytes. Adv Cancer Res 58: 143-175, 1992.

Mosmann TR, Li L, Hengartner H et al. Differentiation and functions of T cell subsets. Ciba Found Symp 204: 148-158, 1997.

Mulligan HD, Beck SA, Tisdale MJ. Lipid metabolism in cancer cachexia. Br J Cancer 66: 57-61, 1992.

Mulligan HD and Tisdale MJ. Metabolic substrate utilization by tumour and host tissues in cancer cachexia. Biochemical Journal 277: 321-326, 1991.

Ollenschlager G, Viell B, Thomas W et al. Tumor anorexia: Causes, assessment, treatment. Rec Result Canc Res 121: 249-259, 1991.

Purasiri P, Ashby J, Heys SD et al. Effect of essential fatty acids on natural cytotoxicity in patients with colorectal cancer. Eur J Surg Oncol 21: 254-260, 1995.

Robins RA. T-cell responses at the host:tumor interface. Biochim Biophys Acta 865: 289-305, 1986.

Robinson LE. In vivo and In vitro Studies of Anticancer Defense: Diet, Exercise and Immunity. 223-229, 2000. 9-1-2000.

Taylor DD, Gercel-Taylor C, Jenis L et al. Identification of a human tumor-derived lipolysis-promoting factor. Cancer Res. 52:829-834, 1992

Tisdale MJ. Wasting in cancer. J Nutr 129: 243S-246S, 1999.

Tissot M, Mathieu J, Mirossay L et al. Polyphosphoinositide metabolism in polymorphonuclear cells from healthy and thermally injured rats: effect of the immunomodulator RU 41740. J Leukoc Biol 50: 607-614, 1991.

Virella G, Fourspring K, Hyman B et al. Immunosuppressive effects of fish oil in normal human volunteers: correlation with the in vitro effects of eicosapentanoic acid on human lymphocytes. Clin Immunol Immunopathol 61: 161-176, 1991.

von Schacky C, Fisher S, Weber PC. Long-term effects of dietary marine n-3 fatty acids upon plasma and cellular lipids, platelet function, and eicosanoid formation in humans. J Clin Invest 76: 1626-1631, 1985.

Wakefield CH, Carey PD, Foulds S et al. Polymorphonuclear leukocyte activation. An early marker of the postsurgical sepsis response. Arch Surg 128: 390-395, 1993.

Waterhouse C and Kemperman JH. Carbohydrate metabolism in subjects with cancer. Cancer Res 31: 1273-1278, 1971.

Wigmore SJ, Fearon KC, Maingay JP et al. Down-regulation of the acute-phase response in patients with pancreatic cancer cachexia receiving oral eicosapentaenoic acid is mediated via suppression of interleukin-6. Clin Sci 92: 215-221, 1997.

Wigmore SJ, Pleater CE, Richardson RA et al. Changes in nutritional status associated with unresectable pancreatic cancer. Br J Cancer 75: 106-109, 1997.

Wigmore SJ, Ross JA, Falconer JS et al. The effect of polyunsaturated fatty acids on the progress of cachexia in patients with pancreatic cancer. Nutrition 12: S27-S30, 1996.

IX.

Changes in Membrane Composition are Associated with Changes in Neutrophil Oxidative Burst in an *In Vitro* Model

A. Introduction

The fatty acid composition of immune cell membranes has been reported to affect cellular function through a variety of mechanisms including changes in membrane fluidity, membrane proteins, intracellular messages and eicosanoid production (reviewed in Section I.F.3). In each human trial (Chapters 3,7,8), there were alterations in the fatty acid composition of neutrophil PL (Sections III, VII, VIII). In particular, the 20:4n-6 content was associated with changes in the oxidative burst and granularity of neutrophils in these studies. Several lines of evidence suggest 20:4n-6 is involved in the oxidative burst activity of human neutrophils. Neutrophils have been reported to have PLA_2 activity that is specific for membrane 20:4n-6 (Meshulam et al.,1992; Ramesha et al.,1993). PLA_2 activity has been reported to be increased upon stimulation of neutrophils (Cockcroft et al.,1989; Mayer et al.,1996). Arachidonic acid has been shown to be involved in oxidative burst signalling pathways of human neutrophil cell lines (Mayer et al., 1996) and has also been reported to be involved in the "priming" response (Mayer et al., 1996). Prostaglandins derived from 20:4n-6 are thought to play a role in the oxidative burst of neutrophils (Bjornson et al.,1989; Takasaki et al.,1996). Arachidonic acid is involved in phagocytosis which activates NADPH oxidase activity (Lennartz,1999).

The objective of this study was to change the 20:4n-6 content of human neutrophil PL to levels similar to those observed in the human trials (Chapters 3, 7, 8) and measure changes in oxidative burst and granularity. An *in vitro* model was developed to determine whether compositional changes in cellular PL observed in these studies were associated with functional changes in neutrophils. Increases in EPA content of cultured cells has been reported to decrease the 20:4n-6 content (Kinsella,1990; Ogle et al.,1990). Linoleic acid, from which 20:4n-6 can be derived, was a major component of the clinical diet fed to the burn subjects. The fatty acids used in this experiment are shown in Table

IX.1. Human primary cell culture was chosen for these experiments as it was thought to be the most physiologically relevant model (Chen et al.,1994; Ramesha et al., 1993) for the proposed objective and application to the clinical studies.

It was hypothesized that a change in the content of 20:4n-6 in neutrophil PL within physiological ranges observed in the human studies, would result in changes to the oxidative burst and granularity of neutrophils. More specifically, increasing the 20:4n-6 content would result in increased oxidative burst and increasing the n-3 content would decrease the oxidative burst.

B. Methods

1. Materials

Tritiated arachidonic acid ([5,6,8,9,11,12,14,15-^{3}H]-arachidonic acid) was purchased from Du Pont New England Nuclear (Boston, MA). Arachidonic, eicosapentaenoic and elaidic acid were purchased as a free fatty acids from Nu-chek Prep (Elysian, MN). The fatty acid 16:0 (hexadecanoic acid) and fatty acid free-BSA were purchased from Sigma (St. Louis, MO). Linoleic acid was extracted from safflower oil (Ma et al.,1999). All other materials were described in Section III.B.1.

2. Ethics approval

The protocol for this study was approved by the Agriculture, Forestry and Home Economics Research Ethics Board. Informed consent was obtained from subjects (n=8) who were recruited from the University of Alberta. Blood (15 ml) was drawn into 10 ml heparinized tubes from the medial cubital vein by a medical laboratory technologist and placed on ice immediately.

[illegible] Preparation of Fatty acids

All procedures were performed under sterile conditions. Stock solutions of fatty acids were prepared in ethanol (10 mg/ml). From the stock solution, fatty acids were serially diluted to a concentration of 0.01M. Prior to the experiments, an aliquot of this stock solution was added to 15 ml conical tubes to achieve a final concentration of 10^{-7} M.

Ethanol was evaporated under nitrogen. Hank's balanced salt solution (HBSS) containing + 0.1% fatty acid free BSA (w/v) was added to each tube and tubes were incubated in a shaking water bath (37°C) for 1 hour with vortexing every 15 minutes. This procedure has been shown to optimize conjugation of fatty acid to BSA (Robinson,2000).

4. Fatty Acid Incorporation

From a time trial fatty acid incorporation experiment (Appendix IX.AP1), it was determined that 4 hours was an optimal timepoint to achieve fatty acid incorporation and maintain oxidative burst in isolated neutrophils. Neutrophils were isolated from whole blood using Ficoll-Hypaque 1119 and 1077 as described previously in Section III.B.6. After removal of the neutrophil band, cells were washed twice with sterile HBSS. Neutrophils were counted using trypan blue exclusion. An aliquot of isolated neutrophils (1×10^6) was added to each experimental tube containing fatty acids conjugated to BSA (20:4n-6, 18:2n-6, EPA, 16:0, 18:1) and one tube containing buffer only. Volume was made up to a total of 2 ml with HBSS + 0.1% (w/v) BSA. When possible all assays were performed in duplicate. Suspensions were incubated in a shaking water bath for 4 hours.

5. Neutrophil Oxidative Burst

After 4 hours incubation, cells were pelleted by centrifuging for 5 minutes at 1250 rpm. Supernatants were removed and cells were washed twice with HBSS. After the final wash, cells were resuspended in sterile 400 µl HBSS + EDTA and aliquots transferred into 4 ml polypropylene tubes for lipid and flow cytometry analysis. DHR (1.8 µl) was added to each tube to be used for flow cytometric oxidative burst analysis as described in Section III.B.8. Cells to be used for lipid analysis were immediately frozen at –20°C. Cells to be used for analysis of neutrophil function were analyzed immediately using flow cytometry as described in Section III.B.8. After acquisition of the cells, the samples were frozen immediately.

6. Lipid Extraction and Phospholipid Analysis

When cell numbers were insufficient to perform both oxidative burst and lipid analysis with the same subject, cells used for flow cytometry analysis were pooled with cells used for lipid analysis to obtain the greatest number of cells possible. The 5 minute and 15 minute stimulated samples were pooled to measure the lipid composition of stimulated cells. A modified Folch procedure was used to extract lipid from the suspensions as described in Section III.B.7. Extracted lipid was made up in 100 µl in chloroform and plated on G-plates to isolate total PL as described in Section V.B.2. Following thin layer chromatography, the band at the origin was scraped into methylation tubes and samples were methylated immediately using BF_3 and hexane as described previously in Section V.B.2. After tubes cooled, 1 ml ddH_20 was added, sample vortexed and held overnight at 4°C. The top layer was removed and dried down under nitrogen. Hexane (100 µl) was added to the dried samples and inserts placed in each GC vial. Fatty acid methyl esters were separated and identified using gas liquid chromatography as described in Section VIII.B.3.

7. Statistical Analysis

Differences in fatty acid composition and oxidative burst between lipid incubations were determined using a paired t-test. For each culture condition, differences in fatty acid composition between unstimulated and stimulated cell cultures was determined using a paired t-test. Correlations between fatty acid composition and oxidative burst were performed using correlational linear regression analysis. Statistical analysis was carried out using the SAS statistical package (Version 6.12).

C. Results

1. Phospholipids Fatty Acid Composition

In the human trials, the major PL fractions rather than total PL were isolated from neutrophils. Based on the percent each major PL comprises in neutrophil cell membranes (Gottfried,1972), calculations were performed to estimate fatty acid composition of total PL in each of these studies. This calculation is based on PC and PE comprising 38.6 ±

0.8%, and 33.4 ± 0.5%, respectively and PS and PI together comprising 15.0 ± 0.5% of membrane PL (Gottfried,1972). Missing from this calculation is the sphingomyelin fraction which comprises 10.5 ± 0.3% (Gottfried,1972). Sphingomyelin composition was not determined in the human studies, however, it would be part of the total PL isolated from neutrophils in this experiment. Therefore values were adjusted to a total of 100% total PL and the estimates of total PL from each study based on the mean content of each fatty acid group from the human trials are shown in Table IX.4.

Culturing human neutrophils in media deplete of fatty acids (buffer group) resulted in 20:4n-6 levels similar to those observed in human studies (TableIX.4) with the exception of the discharge timepoint in the HDCT study (Section VII) and levels observed prior to FO supplementation (Section VIII). The EPA content of cultured neutrophil PL was higher than levels observed in human studies whereas DHA levels were similar to the patient groups studied. Saturated, n-6, and PUFA fatty acid content of neutrophils isolated prior to supplementation with fish oil was not achieved but were similar to levels observed in other patient groups. The MUFA content at harvest and both pre and post fish oil supplementation was less than levels achieved in cell culture. Culturing cells in fatty acid depleted media (buffer only) resulted in higher levels of PUFA and 18:2n-6 than what was observed in burn patients at t1 and t5. Overall, we can conclude that levels of fatty acid incorporation observed in the cell culture experiments were within physiological ranges observed in each of the trials.

2. Relationship of 20:4n-6 to the Oxidative Burst

Neutrophils cultured in EPA had significantly greater content of 20:4n-6 (Table IX.2) and significantly higher 5 minute oxidative burst compared to neutrophils cultured without fatty acids (buffer, Table IX.3). Neutrophils cultured in EPA had significant differences in the 18:2n-6 and EPA content after stimulation compared to cells cultured in buffer (Table IX.2). Neutrophils cultured in EPA had greater 20:4n-6 content compared to the LA group (Table IX.2) and had a significantly greater 5 minute oxidative burst ratio (Table IX.3). After stimulation, cells culture in EPA had significant changes in the MUFA, n-3, EPA and DHA compared to the 18:2n-6 group (Table IX.2).

Neutrophils cultured in buffer exhibited higher n-6 content compared to those cultured in 18:1 and a significantly lower 5 minute oxidative burst. There were significant differences in the n-3 and EPA content after stimulation between these two groups. Culturing cells in 20:4n-6 did not result in significant changes in fatty acid composition or oxidative burst compared to neutrophils cultured with other fatty acids. There were no significant differences observed in the granularity of neutrophils neither before not after stimulation by culturing with any of the fatty acids. The 20:4n-6 content of neutrophil PL did not significantly change with PMA stimulation in this model.

D. Discussion

Culturing human neutrophils with various fatty acids resulted in physiological levels of fatty acids observed in the various human trials that produced subtle changes in neutrophil function. Although granularity did not appear to be affected by changes in cellular composition, the oxidative burst was significantly reduced when 20:4n-6 levels were decreased in PL such as observed in cells cultured with linoleic acid and without fatty acids. Likewise, neutrophils with a higher 20:4n-6 content had higher oxidative burst responses compared to cultures with the lowest 20:4n-6 levels. Together, these results support that 20:4n-6 content of neutrophil PL does have an effect on the oxidative burst of neutrophils. However, the magnitude of change, both in the content of 20:4n-6 and the oxidative burst was not as high as what was observed in the clinical studies, thus additional factors *in vivo* are involved. In these cell culture experiments, the 0 minute (unstimulated) mean fluorescence was higher than what was observed in the human studies. As a result, indexes within the ranges observed in the patient groups studied were not achieved. Cells cultured in buffer (which had the lowest oxidative burst) may have been deplete of other fatty acids necessary for function. However, their response did not differ significantly from the control fatty acids and 4 hours was likely not long enough to induce this. Contrary to other reports (Daniels et al.,1992; Doerfler et al.,1994; Ramesha et al., 1993), there was not a significant change in 20:4n-6 content in the membranes following stimulation in this study. However, those studies did not measure membrane composition directly but rather release of 20:4n-6 into supernatants which would not take into account synthesis or conversion of other fatty acids to 20:4n-6.

The change in arachidonic acid and corresponding oxidative burst differed from the human studies. In both the burn and HDCT trials, lower 20:4n-6 content was associated with higher oxidative burst responses. Achieving levels of 20:4n-6 such as those observed early after burn in some patients and at discharge in the HDCT patients was not achieved by culturing cells in media deplete of fatty acids. In these cell culture experiments, 20:4n-6 content was reduced by eliminating all fatty acids from the media. However, in the disease state, reduction of 20:4n-6 in the membranes likely occurs as a result of biological events of the disease. Changes in 20:4n-6 in PL may impact on the generation of second messengers (Cockcroft et al., 1989; Mayer et al., 1996; Takasaki et al., 1996) that activate the oxidative burst when cells are stimulated *in vitro* (Bass et al.,1986; Garner et al.,1994). The events preceding 20:4n-6 reductions in humans were not included in cell culture system, however, several factors have been shown to play an important role in neutrophil responses *in vivo* such as cytokines, hormones and the presence of infection (Daniels et al., 1992; Doerfler et al., 1994; Rodeberg et al.,1997).

There were no significant differences in n-3 fatty acid content prior to stimulation. However, it does appear that they have some role in the oxidative burst response as supported by the significant reductions in n-3, EPA and DHA content after stimulation. Although 20:4n-6 was the main focus of this investigation, it also appears that 18:2n-6 may also play a role as it was reduced prior to stimulation in the EPA culture which had the highest oxidative burst response. Fatty acids used in these culture models could have other yet to be identified effects on the oxidative burst response of neutrophils.

To summarize, high content of 20:4n-6 in neutrophil PL was associated with higher oxidative burst with stimulation. Although reduced 20:4n-6 in human studies were associated with higher oxidative burst responses, the events preceeding low 20:4n-6 levels may play an important role in neutrophil activation. Significant changes in content of fatty acids occur after stimulation *in vitro* and support a role of n-3 fatty acids in oxidative burst responses. Dissecting the complex relationships that are apparent between fatty acid compositions and cellular functions, particularly in disease states requires further investigation.

Table IX.1

Fatty Acid	Rationale
20:4n-6[a]	To increase the 20:4n-6 content of the membrane through direct incorporation of 20:4n-6 into membrane PL.
EPA[a]	To increase EPA in the membrane such as that proposed by feeding fish oil to cachexic cancer patients (Chapter VII) and to inhibit 20:4n-6 metabolism and replace it in cellular PL (Gerster,1995).
18:2n-6[b]	To determine if 18:2n-6 incorporation into membranes has similar effects to 20:4n-6
18:1[a]	To serve as a fatty acid control
16:0[c]	To serve as a fatty acid control
Buffer[d]	to reduce 20:4n-6 content in neutrophil PL

[a] free fatty acids purchased from Nu-chek Prep (Elysian, MN).

[b] extracted from safflower oil (Ma et al., 1999)

[c] Sigma (St. Louis, MO)

[d] HBSS + fatty acid free BSA

TABLE IX.1: Fatty acids used in the cell culture experiments.

Table IX.2

		20:4n-6	EPA	18:2n-6	18:1	16:0	Buffer
ΣSFA (%)	**US**	48.1 ± 2.7^{a}	44.1 ± 1.3^{ab}	46.4 ± 1.5^{ab}	47.1 ± 3.7^{ab}	44.6 ±0.8^{ab}	40.6 ± 2.1^{b}
	ΔS	-2.0 ± 3.8	-1.0 ± 3.5	-5.3 ± 3.8	2.0 ± 1.3	n/a	-5.9 ± 4.3
ΣMUFA (%)	**US**	26.5 ± 1.5	26.9 ± 1.3	29.0 ± 0.7	27.6 ±1.4	27.0 ±1.1	26.6 ± 1.7
	ΔS	-1.2 ± 2.0^{ab}	-3.7 ± 1.6^{a}	3.2 ± 1.8^{b}	0.6 ± 2.2^{ab}	n/a	2.0 ± 2.3^{b}
ΣPUFA (%)	**US**	25.4 ± 3.4	29.0 ± 2.2	24.6 ± 1.6	25.3 ± 4.5	28.0 ± 1.1	32.8 ± 3.4
	ΔS	4.3 ± 5.1	3.6 ± 4.7	3.0 ± 5.1	-2.6 ±5.7	n/a	3.9 ± 5.7
ΣN-6 (%)	**US**	21.0 ± 3.5^{a}	25.2 ± 2.8^{ac}	19.7 ± 1.4^{ab}	21.1 ± 4.1^{a}	22.3 ± 1.0^{a}	27.4 ± 2.9^{bc}
	ΔS	3.6 ± 4.8	5.2 ± 4.4	-0.2 ± 4.8	-3.9 ± 5.4	n/a	3.5 ± 5.4
ΣN-3 (%)	**US**	4.5 ± 0.8	3.9 ± 0.8	4.9 ± 0.71	4.7 ± 0.6	5.8 ± 0.9	5.3 ± 0.5
	ΔS	0.8 ± 1.2^{ab}	-2.6 ± 0.8^{c}	3.2 ± 1.7^{b}	3.1 ± 1.3^{b}	n/a	-0.5 ± 1.0^{ac}
18:2n-6 (%)	**US**	6.1 ± 1.8^{a}	7.7 ± 1.5^{a}	8.6 ± 1.7^{a}	12.3 ± 3.9^{ab}	10.77 ±1.96^{ab}	21.0 ± 2.0^{b}
	ΔS	2.7 ± 4.1^{a}	-4.0 ± 3.8^{b}	2.2 ± 4.1^{ab}	-2.5 ± 4.6^{ab}	n/a	9.0 ± 4.6^{a}
20:4n-6 (%)	**US**	13.4 ± 2.1^{ac}	13.3 ± 1.7^{a}	7.6 ± 2.0^{bc}	9.17 ±1.7^{ab}	10.04 ±2.29^{ab}	6.7 ± 2.3^{b}
	ΔS	1.0 ± 4.6	9.7 ± 4.1	-1.4 ± 4.6	0.9 ± 5.2	n/a	-0.02 ± 5.2
EPA (%)	**US**	2.8 ± 0.3	2.2 ± 0.3	2.8 ± 0.3	2.8 ± 0.3	3.0 ± 0.5	2.5 ± 0.3
	ΔS	-0.1 ± 0.4^{a}	-0.6 ± 0.4^{b}	0.9 ± 0.4^{ac}	-1.9 ± 0.5^{bc}	n/a	0.02 ± 0.5^{a}
DHA (%)	**US**	0.8 ± 0.3	0.7 ± 0.3	0.7 ± 0.3	0.4 ± 0.2	1.3 ± 0.6	0.6 ± 0.4
	ΔS	0.4 ± 0.4^{ab}	-0.5 ± 0.4^{a}	0.8 ± 0.4^{b}	0.2 ± 0.5^{ab}	n/a	-0.5 ± 0.5^{a}

TABLE IX.2: Fatty acid composition of cells following incubation with fatty acids (shown in headings) for 4 hours is shown in the shaded rows. Changes in fatty acid groups (shown on the left side) after stimulation with PMA *in vitro* are shown in clear rows. Data is shown as mean ± SEM. Significant differences ($p<0.05$) in fatty acid groups between culture conditions were determined using a paired t-test ($n \le 8$).

Abbreviations: US=unstimulated, ΔS=change after stimulation, EPA=eicosapentaenoic acid, SFA=saturated fatty acids, MUFA=monounsaturated fatty acids, PUFA= polyunsaturated fatty acids, DHA=docosahexaenoic acid

Table IX.3

Oxidative Burst	*20:4n-6*	*EPA*	*18:2n-6*	*18:1*	*16:0*	*Buffer*
			(Mean Channel Fluorescence)			
0 min	74 ± 8^{ab}	73 ± 7^{ab}	86 ± 7^{b}	83 ± 9^{ab}	80 ± 9^{ab}	56 ± 8^{a}
5 min	303 ± 33^{ab}	359 ± 32^{a}	367 ± 32^{ab}	406 ± 37^{ac}	275 ± 45^{bc}	205 ± 37^{b}
5 index	4.3 ± 0.4^{ab}	5.2 ± 0.4^{b}	4.0 ± 0.4^{a}	5.2 ± 0.7^{b}	3.5 ± 0.5^{ab}	3.6 ± 0.4^{a}
15 min	805 ± 66	872 ± 63	896 ± 63	1080 ± 71	934 ±95	787 ± 73
15 index	11.1 ± 1.0	12.9 ± 1.0	10.4 ± 1.0	13.6 ± 1.0	13.6± 0.6	12.7 ± 1.1

TABLE IX.3: Oxidative burst and oxidative burst ratios of neutrophils cultured with fatty acids. Gradient isolated neutrophils were incubated with fatty acids for 4 hours. Cells were incubated with DHR and stimulated with PMA for 5 and 15 minutes. The oxidative burst was measured immediately using flow cytometry. The oxidative burst ratio is given by the formula: stimulated FL-1 at 5, 15 minutes/FL-1 at 0 minutes. Differences ($p<0.05$) in oxidative burst obtained with different fatty acid cultures were determined using a paired t-test (n=8).

Table IX.4

Fatty Acid	*T1 Burns*[a]	*T5 Burns*	*HDCT Discharge*[b]	*HDCT Harvest*	*Pre FO*[c]	*Post FO*	*Range of Cultured Cells*
				(% fatty acids)			
ΣSFA	40.5	38.3	48.6	37.5	27.3	30.8	41-48
ΣMUFA	30.7	25.8	31.4	23.8	21.5	25.2	27-29
ΣPUFA	19.3	27.5	33.4	24.5	31.0	24.2	25-32
ΣN6	15.3	16.7	30.4	22.5	29.7	23	19-27
ΣN3	4.5	7.8	2.9	2.0	1.3	1.3	4-6
18:2n6	7.0	6.3	20.7	14.1	8.0	10.2	6-21
20:4n-6	6.9	12.2	7.4	4.2	16.6	13.9	7-13
EPA	1.4	0.72	0.97	0.71	0.18	0.21	2.2-3.0
DHA	0.63	1.08	0.29	0.11	0.74	0.72	0.4-1.3

[a] Section III

[b] Section VII

[c] Section VIII

TABLE IX.4: Total PL in the human trials (based on group means) and calculated based on the percent each major PL fraction constitutes in neutrophils (Gottfried,1972).

E. References

Bass DA, Olbrantz P, Szejda P et al. Subpopulations of neutrophils with increased oxidative product formation in blood of patients with infection. J Immunol 136: 860-866, 1986.

Bjornson AB, Knippenberg RW, Bjornson HS. Bactericidal defect of neutrophils in a guinea pig model of thermal injury is related to elevation of intracellular cyclic-3',5'-adenosine monophosphate. J Immunol 143: 2609-2616, 1989.

Chen LY, Lawson DL, Mehta JL. Reduction in human neutrophil superoxide anion generation by n-3 polyunsaturated fatty acids: role of cyclooxygenase products and endothelium-derived relaxing factor. Thrombosis Research 76: 317-322, 1994.

Cockcroft S and Stutchfield J. The receptors for ATP and fMetLeuPhe are independently coupled to phospholipases C and A2 via G-protein(s). Relationship between phospholipase C and A2 activation and exocytosis in HL60 cells and human neutrophils. Biochem J 263: 715-723, 1989.

Daniels RH, Finnen MJ, Hill ME et al. Recombinant human monocyte IL-8 primes NADPH-oxidase and phospholipase A2 activation in human neutrophils. Immunol 75: 157-163, 1992.

Doerfler ME, Weiss J, Clark JD et al. Bacterial lipopolysaccharide primes human neutrophils for enhanced release of arachidonic acid and causes phosphorylation of an 85-kD cytosolic phospholipase A2. J Clin Invest 93: 1583-1591, 1994.

Garner WL, Rodriguez JL, Miller CG et al. Acute skin injury releases neutrophil chemoattractants. Surg 116: 42-48, 1994.

Gerster H. The use of n-3 PUFAs (fish oil) in enteral nutrition. Int J Vitam Nutr Res 65: 3-20, 1995.

Gottfried EL. Lipid patterns of leukocytes in health and disease. Sem Hematol 9: 241-250, 1972.

Kinsella JE. Lipids, membrane receptors, and enzymes: effects of dietary fatty acids. JPEN J Parenter Enteral Nutr 14: 200S-217S, 1990.

Lennartz MR. Phospholipases and phagocytosis: the role of phospholipid-derived second messengers in phagocytosis. Int J Biochem Cell Biol 31: 415-430, 1999.

Ma DW, Wierzbicki A, Field CJ et al. Preparation of conjugated linoleic acid from safflower oil. JAOCS 76: 729-730, 1999.

Mayer AM, Brenic S, Glaser KB. Pharmacological targeting of signaling pathways in protein kinase C-stimulated superoxide generation in neutrophil-like HL- 60 cells: effect of phorbol ester, arachidonic acid and inhibitors of kinase(s), phosphatase(s) and phospholipase A2. J Pharm Exp Ther 279: 633-644, 1996.

Meshulam T, Herscovitz H, Casavant D et al. Flow cytometric kinetic measurements of neutrophil phospholipase A activation. J Biol Chem 267: 21465-21470, 1992.

Ogle CK, Wu JZ, Wood S et al. The increased release of prostaglandin E2 by Kupffer cells from burned guinea pigs. J Burn Care Rehab 11: 287-294, 1990.

Ramesha CS and Ives DL. Detection of arachidonoyl-selective phospholipase A2 in human neutrophil cytosol. Biochim Biophys Acta 1168: 37-44, 1993.

Robinson LE. In vivo and In vitro Studies of Anticancer Defense: Diet, Exercise and Immunity. 223-229, 2000. 9-1-2000.

Rodeberg DA, Bass RC, Alexander JW et al. Neutrophils from burn patients are unable to increase the expression of CD11b/CD18 in response to inflammatory stimuli. J Leukoc Biol 61: 575-582, 1997.

Takasaki J, Kawauchi Y, Yasunaga T et al. Human type II phospholipase A2-induced Mac-1 expression on human neutrophils. J Leukoc Biol 60: 174-180, 1996.

X.

General Summary and Discussion

A. General Summary

1. The Effects of Burn injury on Phospholipid Composition and Function of Immune Cells

It was hypothesized that:

a) the phospholipid fatty acid composition of immune cells, lymphocytes and neutrophils, will be altered after burn injury and change in the direction of healthy individuals during recovery of the patient. More specifically:

(i) The phospholipids of lymphocytes and neutrophils will have a lower content of arachidonic acid immediately post-injury in the major phospholipids fractions and increase with recovery.

Each major PL fraction of neutrophils and lymphocytes exhibited reduced amounts of 20:4n-6 at the first post burn timepoint compared to the final sampling timepoint and compared to healthy subjects (Figures III.2 and IV.2; Table III.7). By the last sampling timepoint (>50 days post burn), the 20:4n-6 content was similar to levels observed in healthy individuals. The length of time it took for significant increases in 20:4n-6 to occur differed among the major PL fractions and between neutrophils and lymphocytes. The PE and PI fractions of lymphocytes doubled in 20:4n-6 content after t1, whereas this magnitude of change was not observed in any neutrophil PL fraction.

(ii) Total n-6 and n-3 fatty acids in the phospholipids of lymphocytes and neutrophils will be reduced in the early post burn period and will increase during recovery in the direction of that seen in the healthy population.

The change in the concentration of these lipid classes varied among major PL fractions and lymphocytes and neutrophils (Tables III.9; IV.8-11). Few changes in total n-3 content were observed in neutrophils whereas n-3 content tended to be higher in lymphocytes at the early compared to later post burn timepoints. Conversely, the n-6 content was lower at the initial postburn timepoints and increased with recovery in both lymphocytes and neutrophils. The changes in n-3 fatty acids were not attributed to a specific fatty acid.

b) There will be impaired function of immune cells of both the acquired and innate immune branches post-burn injury. More specifically:

(i) After burn, the relative percentages of CD4 cells will be low.

Percentage of CD4 cells was not reduced post injury but rather it was the CD8 cells that were reduced early after burn and this phenotype increased with recovery (Table IV.3).

(ii) Cell mediated immunity, as estimated by mitogen stimulated [^{3}H]-thymidine incorporation, will be decreased at 1-12 days post injury and will increase as the patient recovers. Unstimulated [^{3}H]-thymidine incorporation will be high initially and decrease with recovery.

The unstimulated mitogen response was highest at 12-19 days post burn (Table IV.6). Mitogen stimulated [^{3}H] thymidine incorporation was lowest at t1 in response to PMA and lowest at t2 in response to Con A. Therefore an increased *in vivo* activation and a reduced ability to respond to mitogenic challenges were observed within the first 21 days post burn injury. The unstimulated and stimulated responses at 21-35 days did not differ from the later timepoints.

(iii) The TH1 subset will be activated immediately post-injury with a high production of IFN-γ and IL-2.

The production of cytokines produced by the TH1 subset of lymphocytes was not statistically different between post burn timepoints (Section IV.C.5). However, other changes in lymphocyte function (increased expression of CD25 and CD71 on $CD8^+$ cells, higher unstimulated proliferative response, lower CD45RO/CD45RA ratio, increased natural killer cytotoxicity) and fatty acid composition support a change in T-cell phenotypes at 12 –19 days post burn injury (Table IV.6, Section IV.D).

(iv) T cells (CD4+ and CD8+) will express a high percentage of CD45RA (antigen naivity) in the initial post burn period and the percent CD45RO (antigen mature) will increase by the second timepoint (d12-19). Activation markers (CD25 and CD71) on lymphocytes will be high early after burn and decrease with recovery.

The highest expression of CD45RO was observed at 21-35 days post burn and the highest expression of CD45RA was observed at 12-35 days post burn which were both significantly higher than levels observed at >50 days post burn (Table IV.3). There were no significant differences in the expression of CD45RO and CD45RA on the CD4 and CD8 subsets (Table IV.4). However, the ratio of CD45RO/CD45RA cells on CD4 cells was reduced at 12-19 days post injury (Table IV.4). The total expression of CD71 and CD25 was not different between post burn timepoints, however, $CD8^+$ cells expressed higher levels of CD25 and CD71 at 12-19 days compared to 21-35 days post burn (Table IV.5).

(v) Natural Killer cell activity will be low initially following burn injury and increase with recovery.

Even though numbers of NK cells did not change, a significant increase in NK cell cytotoxicity was observed at 12-19 days compared to <12 days (Table IV.3, Figure IV.1). Number of NK cells was lowest at >50 days post burn and the cytotoxicity was highest at this timepoint suggesting that there was a reduced cellular activity in the early post-burn period.

(vi) Neutrophil activation will be high initially producing high amounts of oxygen radicals during the first weeks following burn. Unstimulated neutrophil activity will decrease as the patient recovers.

The unstimulated responses of neutrophils did not significantly change throughout recovery from burn injury. Neutrophils isolated between 12 and 19 days exhibited the highest stimulated oxidative burst response (Figure III.1) with values approaching 3 times higher than healthy subjects. These results suggest that neutrophils isolated between 12 and 19 days post burn may have been primed *in vivo* for a higher oxidative burst response. The level of this response was not observed in any other patient group.

(vii) The proportion of macrophages (particularly activated macrophages) will be high at the first sample point post-burn injury.

The highest proportion of activated macrophages was observed at 12-19 days post injury and this decreased with recovery (Table IV.3). Macrophages are a source of prostaglandins and inflammatory cytokines and this study supports the literature that there is an increase in these mediators early after burn.

2. Effects of Burn injury on fatty acid composition of plasma components and erythrocytes

It was hypothesized that:

a) the 20:4n-6 content of RBCs, CE, TG and PL will be reduced following burn injury and increase with recovery.

The percent 20:4n-6 in plasma CE and PL was reduced in the early post burn period compared to later post burn timepoints (Table V.1, V.4). On quantitative basis, 20:4n-6 was reduced in all plasma fractions measured at the first timepoint compared to healthy control subjects. The 20:4n6 content increased with recovery, both on a percentage and quantitative basis to levels observed in healthy individuals in the TG and PL fractions. However, 20:4n-6 remained significantly less on a quantitative basis in the CE fraction even >50 days post burn. The 20:4n-6 content of RBC PL was lower at t1 compared to t5 in all fractions except PE (Tables V.8, V.10, V.11). These results suggest increased utilization/release or reduced synthesis of 20:4n-6 after burn injury.

b) there will be reduced concentrations of CE and PL and elevated concentrations of TG in plasma early post burn that return to normal levels (those observed in healthy individuals) with recovery from injury.

The concentrations of PL and CE in the plasma of burn patients were significantly lower at 0-12 days than at >50 days post burn and concentrations observed in healthy individuals (Figure V.1, V.2; Table V.1, V.4). Hypertriglyceridemia was not observed at any timepoint, but rather reduced levels of TG compared to healthy controls was also observed at the early post burn timepoints (Table V.6). CE levels did not return to levels observed in healthy controls even after 50 days post burn (Table V.4). These results suggest that are alterations in the synthesis or a change in the distribution of lipoproteins following burn injury.

3. The Effects of Surgery and Infection on Immune Function in Guinea Pigs

It was hypothesised in a guinea pig model that:

a) surgery will result in reductions in the oxidative burst activity of neutrophils, decreased natural killer cell cytotoxicity, reduced mitogen stimulated [^{3}H]-thymidine uptake, and a reduced CD4/CD8 ratio in lymphoid

tissues.

Compared to control animals, animals undergoing surgery had depressed NK cell activity (Figure VI.2) and lower mitogen reponses (Table VI.2). Animals undergoing surgery exhibited no change in the splenocyte CD4/CD8 ratio and had similar oxidative burst responses by neutrophils compared to the control animals (Table VI.4). Neutrophils from noninfected pigs were larger and less granular than control animals (Table VI.4). These results confirm the immunosuppressive effects of surgery.

b) infection with *Staphylococcus aureus* post surgery will result in an increased oxidative burst of neutrophils, increased MHC Class II molecule expression on immune cells and a further (compared to the effects of surgery alone) depression of NK cytotoxicity and mitogen stimulated [^{3}H]-thymidine uptake.

Animals infected with *S. aureus* had increased unstimulated and stimulated oxidative burst by neutrophils (Table VI.4) and higher splenocyte [^{3}H]-thymidine uptake in response to PWM (Table VI.2). Incorporation of [^{3}H]-thymidine did not differ from the noninfected group for any other mitogen. Infected animals had a further depression of NK activity compared to the noninfected animals (Figure VI.2). However, unlike the proposed hypothesis, MHCII expression was reduced on antigen presenting cells in the spleen (Figure VI.1) but not in the prescalpular lymph nodes (Table VI.3). Therefore, localized infection following surgery can stimulate certain immune parameters while depressing others.

c) infected animals treated with the Acticoat™ Silver coated dressing will have immune responses similar to the noninfected surgery animals.

Acticoat treated animals had similar NK cytotoxicity to noninfected animals (Figure VI.2). However, they had a decreased CD4/CD8 ratio and MHCII expression on antigen presenting cells in the prescalpular lymph nodes compared to both noninfected and infected groups (Table VI.3). Neutrophils from guinea pigs treated with Acticoat similar oxidative burst responses but were smaller and less granular than those from infected pigs (Table VI.4). Therefore, treatment of an infected wound with Acticoat can overcome some but not all, immune parameters depressed by infection.

4. The Effect of Stem Cell Harvest and High Dose Chemotherapy on Immune Function in Women With Breast Cancer

It was hypothesized that:

a) At discharge, cellular function will be reduced from pre-HDCT levels. Specifically, compared to harvest values the following measures will be different at discharge:

(i) The CD4/CD8 ratio will be reduced.

The CD4/CD8 ratio was reduced after the HDCT procedure (Figure VII.2), suggesting depressed cellular immunity after the HDCT treatment

(ii) Neutrophil oxidative burst will be reduced.

Contrary to the hypothesis, the oxidative burst of neutrophils increased after HDCT (Figure VII.3). This was associated with changes in size (Figure VII.4) and granularity in the direction observed in other patient groups. This suggests that neutrophil function is reduced prior to HDCT and improved after the therapy.

(iii) T cells will have lower expression of activation markers.

There were no significant differences in the expression of CD25 or CD71 on lymphocytes between harvest and discharge timepoints.

(iv) The expression of the CD45RO isotype on T-lymphocytes (CD4+ and CD8+) will be decreased.

Contrary to the hypothesis, the expression of CD45RO was increased on lymphocytes following the procedure due to a higher percent of $CD4^+$ cells expressing CD45RO. This suggests that although proportions of CD4 cells were reduced, they may have maintained their ability to produce and respond to cytokines. Further functional measures are required.

b) Immune cell membranes and plasma lipid components will suggest deficiencies in essential fatty acid metabolism both at harvest and at discharge. These deficiencies will be more pronounced following the high dose procedure.

The fatty acid composition of plasma components (pre- and post-HDCT) did not markedly differ from that of control subjects, however, there were reduced concentrations of CE and PL both prior to and after the procedure compared to control subjects (Table VII.4). The percent of n-3 fatty acids was lower at harvest than at discharge and compared to healthy control subjects on a quantitative basis whereas the n-3 content in TG appear to be higher than levels observed in healthy subjects (Table VII.4). The percent of 20:4n-6 in neutrophil PL were markedly reduced compared to control subjects but only the PS fraction exhibited a significant decrease at discharge compared to harvest (Table VII.3). Levels of n-3 fatty acids in PL fractions were generally lower than levels observed in healthy subjects and were significantly reduced in the PS fraction following the HDCT procedure (Table VII.2). These results suggest that alterations in lipid metabolism occur as a result of induction chemotherapy protocols as well as the HDCT procedure.

5. The effects of Fish Oil supplementation on Immune Functions in Cachexic cancer patients

It was hypothesized that:

a) Supplementing weight losing cancer patients with 18 g of long chain polyunsaturated n-3 fatty acids for 14 d, compared to a placebo oil will result in an increase in long chain n-3 fatty acids in the membranes of neutrophils and in the plasma phospholipids.

There was a significant increase in EPA and DHA in plasma after 14 days of supplementation with fish oil on a percent basis (Table VIII.2). However, supplementation did not result in higher levels of these fatty acids in the PL of neutrophils in all subjects. This suggests weight losing cancer patients are able to absorb supplemental n-3 fatty acids but a problem may exist in their ability to incorporate them into cellular membranes.

b) Supplementing palliative cancer patients with 18 g of long chain polyunsaturated fatty acids/d for 14 d, compared to a placebo will improve some peripheral blood measures of immune function (parameters of the acquired and innate immune system). Specifically:

(i) the CD4/CD8 ratio will increase.

The CD4/CD8 ratio did not increase with fish oil supplementation (Table VIII.7).

(ii) the expression of CD45RO on CD4+ and CD8+ T cells will increase.

The expression of CD45RO on CD4 and CD8 cells did not change with fish oil supplementation (Table VIII.7).

(iii) the expression of the IL-2 receptor and CD28 on immune cells will increase.

The expression of CD25 on $CD8^+$ cells increased, and the expression of CD28 on $CD8^+$ cells decreased after 14 days of supplementation with fish oil (Table VIII.7). This suggests that CD8 cell function is altered but the clinical consequence requires further investigation of functional indices of these specific cells.

(iv) The neutrophil oxidative burst will decrease.

The neutrophil oxidative burst did not change with fish oil supplementation (Table VIII.6). However, the unstimulated response was lower after 14 days of fish oil supplementation compared to the group receiving placebo. This suggests a change in the *in vivo* activation state of neutrophils with fish oil supplementation.

6. Changes in phospholipid composition of neutrophils impacts on their functional capacity

It was hypothesized that:

1. Increasing the 20:4n-6 content in neutrophil PL will increase the oxidative burst of neutrophils.

Neutrophils with a higher content of 20:4n-6 had higher oxidative burst responses than those with lower 20:4n-6 content (Table IX.2 and IX.3).

2. Increasing the n3 fatty acid content of neutrophil PL will decrease the oxidative burst of neutrophils.

The cell culture system did not significantly change the content of n-3 fatty acids of neutrophils (Table IX.2). However, significant reductions in the content of n-3 fatty acids in neutrophil PL occurred after stimulation (Table IX.2).

B. Discussion and Future Directions

Several studies have demonstrated immunological alterations in both cell-mediated and innate immune branches following burn injury, and during cancer and chemotherapy. These alterations can lead to immunosuppression or immune activation, either of which can be detrimental to the host. Several of these functions have been shown to relate to fatty acid composition of the immune cells, however, this is the first report, to our knowledge, of changes in membrane composition in immune cells regulating these key effector functions. The changes observed in the fatty acid composition of immune cell membranes may relate to alterations in immune parameters measured in these studies and those reported by others in the literature.

1. The Effects of Burn Injury on Fatty Acid Metabolism and Immune Function

Our study demonstrated decreases in 20:4n-6 content in the PL of lymphocytes, neutrophils and red blood cells, as well as in plasma cholestryl esters, triglycerides and phospholipids following burn injury. In addition, all plasma components and some cellular PL fractions exhibited low levels of n-6 and n-3 fatty acids and higher levels of MUFA and SFA in the early post burn period. Therefore, enzymatic and biochemical alterations involved in the regulation of essential fatty acid metabolism appear to be a whole body response to stress induced by burn injury. The reduction in 20:4n-6 in plasma and immune cell PL appears to occur very rapidly following the injury. These changes cannot then, be entirely attributed to reduced availability from the diet. Increased utilization by peripheral tissues (i.e. skin) and immune cells also likely contribute to the low levels of fatty acids found in plasma.

Lymphocytes have been reported to preferentially oxidize PUFA (Yaqoob et al.,1994) which were reduced in cellular PL following burn injury. Prostaglandin production has been reported to increase following burn injury but the release of 20:4n-6 from PL following burn injury and the relationship to PG production has not been investigated. This study suggests that the decreased level (or increased release of 20:4n-

6) from both neutrophils and lymphocytes may serve as potential sources of eicosanoids derived from 20:4n-6. This change in fatty acid composition seems to be specific for 20:4n-6, as it occurred independently of the 18:2n-6 content, other n-6 fatty acids or n-3 fatty acids. The change in 20:4n-6 was also observed in RBCs, which are frequently used as an indice of essential fatty acid metabolism (Stanford et al.,1991). Increases in the 20:4n-6 content of lymphocytes occurred at the same time as changes in phenotypes and proliferative responses were observed that would further support an increased *in vivo* response in the period post-injury. However, the higher neutrophil responses occurred when 20:4n-6 content was low, which may have also contributed to the lower levels of 20:4n-6 in these cells. Uncontrolled or magnified activation of neutrophils and macrophages may be harmful to the host due to increased damage from free radicals. However, activation of lymphocytes is likely beneficial for infection defenses. The enhanced stimulated oxidative burst response of neutrophils between 12 and 19 days post injury was not observed in any other patient group. The *in vitro* work (Chapter IX) demonstrated a relationship between the oxidative burst of neutrophils to their PL content of 20:4n-6. However, the magnitude of change in neutrophil function was much less than observed in burn patients, suggesting other factors contribute to this response *in vivo*. Arachidonic acid has been shown to be involved in pathways leading to NADPH activation (Cockcroft et al.,1989; Mayer et al.,1996), however, the role of fatty acids in contributing to this response post burn has not been investigated. This warrants further investigation as acute respiratory distress syndrome is currently the major cause of death in burn patients (Hansbrough et al.,1996) and is associated with neutrophil infiltration and activation in lung tissues (Fujishima et al.,1995). Furthermore, investigations into the mechanism by which EFA exert effects on neutrophils would be of benefit in the application to other inflammatory diseases.

Immune responses carried out by CD8 cells following burn injury have not been as well characterized as the CD4 subset of lymphocytes. Our studies suggest alterations in CD8 cells, evidenced by increased expression of activation markers during the early phases of recovery from burn. Like CD4 cells, CD8 cells have been shown to produce different patterns of cytokines (Mosmann et al.,1997). The reports of inhibition of TH1 responses (cytokines also produced by TC1) reported by others (Lyons et al.,1997;

O'Sullivan et al.,1995) and supported by the observations in Chapter IV, deserves further attention.

Although not investigated in these studies, it is likely that the gastrointestinal tract and its associated lymphoid tissues play a role in the changes observed in fatty acid metabolism following burn injury. Absorbed 20:4n-6, like that which occurs in other cell types, is stored in PL of enterocytes (Hollander et al.,1991). The intestinal mucosa has the ability to desaturate and elongate essential and nonessential fatty acids (Thomson et al.,1988). Prostaglandins have been consistently demonstrated to have an important role in the mucosal defense and repair (Hollander et al., 1991; Tao et al.,1984; Wilson et al.,1976; Wilson,1991). Mucosal damage during cold stress has been shown to be inversely proportional to the amount of 18:2n-6 in the diet of rats (Hollander et al., 1991). Burn injury has been shown to induce physiological changes in the gastrointestinal mucosa (Bragg et al.,1991; Hosoda et al.,1989), which has been reported to increase the susceptibility of patients to bacterial translocation from the gut (Maejima et al.,1984). This can result in sepsis, a major threat following burn injury. Therefore, protection offered by PG may serve a potential therapeutic role in the stress response to burn injury. Given the important role of the gut as both a major immune organ and its importance in delivering nutrients to the tissues, the effects of burn injury on the gastrointestinal tract cannot be overlooked. The liver is also an important organ in distributing fatty acids throughout the body. The reduction in plasma concentrations of lipoprotein constituents would suggest liver involvement in the metabolic changes observed in essential fatty acid metabolism following burn injury.

The skin has a large requirement for essential fatty acids (Ziboh et al.,2000) re and the demand would be significantly increased in a burn patient. Clinical signs of essential fatty acid deficiencies are manifest in the skin (Burr et al.,1930). Linoleic acid is the most abundant fatty acid in the skin (Chapkin et al.,1986) and may play a role in the maintenance of the epidermal water barrier. Additionally, 20:4n-6 is the second most abundant PUFA in the skin comprising approximately 9% of total fatty acids in the epidermis (Ziboh et al., 2000). Skin cells lack desaturase enzymes, therefore they must obtain 20:4n-6 from endogenous sources. The mechanism of how they obtain fatty acids from circulation is not well-characterized (Ziboh,1994). Arachidonic acid metabolites have been shown to regulate proliferative and differentiation processes in the epidermis, serving an important role in wound healing. The implications of skin involvement in altering fatty acid availability in response to large burns has not been investigated.

The use of enteral compared to parenteral routes of nutrient administration has proven efficacy (Alverdy et al.,1985; Kudsk et al.,1992; Minard et al.,1994; Moore et al.,1992). In our study, feeding began immediately upon admission, which has been shown to be beneficial in the critically ill patient (Hansbrough,1998). Immune parameters that relate to *in vivo* immune responses were improved after the first 10 days in this patient group. Improved NK cytotoxicity, proliferative responses and immune cell phenotypes, in addition to the low mortality rates observed collectively in patients admitted to this particular burn unit, would support the use of early enteral nutrition containing a high nitrogen content, supplemental vitamin C and medium chain triglycerides. However, the optimal diet for burn patients may not be adequately defined at present. Doing this will require carefully controlled clinical trials to integrate positive results reported in the literature with what is known about immune function, essential fatty acid metabolism, and other nutrients thought to have immune-enhancing properties, rather than adding several different "immuno-nutrients" to an existing formula and measuring outcomes. The few studies that have examined low fat regimens on clinical outcome have shown beneficial effects, regardless of the composition (Garrel et al.,1995; Gottschlich et al.,1990). The impact of low fat diets on the availability of essential fatty acids has not been investigated in burn patients. The beneficial effects of n-3 fatty acids are more pronounced when they are included in low fat regimens (Robinson,2000). The formula used in the present study did not contain carnitine, which has been shown to be deficient early after burn injury and serves an important role in fat metabolism (Harris et al.,1982). Glutamine should also be considered for addition into specialized formulation for burn patients, as it is a major fuel for the gastrointestinal tract and immune cells, and is considered to be an essential amino acid in states of stress (Pratt et al.,2000).

2. Phospholipids

Phospholipid membranes are comprised of unequal proportions of PC, PE, PI and PS. Each of these fractions differs in their relative content of n-3 fatty acids and 20:4n-6. These studies support the work of others (Clandinin et al.,1991; Field et al.,1989; Robinson,2000), that release and incorporation or synthesis of various fatty acids differs in each major PL fraction exemplifying the complexities of membrane structure. Each PL fraction may serve different functional roles in immune cells as indicated by their various and different response to burn injury chemotherapy and cancer. Future studies examining the role of membrane composition in cellular function must isolate these fractions.

3. Surgery and infection

Chapter VI supports the numerous studies that have reported suppressive effects of surgery on innate and cell mediated immune functions. Surgery is an integral and necessary component in the treatment of cancer and burns. Localized infection was shown to stimulate certain aspects of immune function that would be expected to enhance defense against infectious organisms introduced at a local site, such as a wound. Release of cytokines in a localized area serve to enhance or down-regulate specific immune responses. Cytokine production has been demonstrated to be under partial control of fatty acids (Caughey et al.,1996; Endres,1996; Hubbard et al.,1993; Meydani,1992). The role of dietary fat in altering the cytokine profiles of T cells would be of benefit to a wide variety of diseases including burns, cancer and infection. In addition, although not part of these investigations, the prevalence of antibiotic resistant bacteria is increasing and has become a major issue in the care of critically ill patients. Ways in which to reduce the need for the use of antibiotics against these life-threatening bacteria are worthy of further investigation.

4. High Dose Chemotherapy

Very little data exists regarding immune function and fatty acid metabolism during administration of high dose chemotherapy regimens for cancer, making comparisons from our study to the literature not possible. The treatment strategy discussed in Chapter VII was not as immunologically devastating as was predicted, however, significant alterations in essential fatty acids compared to healthy individuals were observed both during the induction chemotherapy period and following the HDCT procedure. These effects could be a cumulative result of long term chemotherapy regimens as well as a reduction in food intake in these patients. The dietary intake of patients undergoing this or other types of high dose procedures has not been documented. Unlike patients with burns, where energy requirements and macronutrient intakes have been more clearly defined, it has been stated that "nutrition during chemotherapy and other cancer treatments are of little benefit" (McGeer et al.,1990). Nutrition in the past has provided in the form of TPN. Not surprisingly, this form of therapy in an immunosuppressed patient has been associated with an increased rate of infection. It may be that previous studies have not tested the appropriate formula that would be aimed at reversing the lipid abnormalities in patients undergoing chemotherapy. Given the impact chemotherapy and other cancer treatments have on immunity and nutritional status, formulations that improve nutritional status, encourage repair of tissues and enhance immune function are clearly warranted in this group of patients, not only during acute inpatient procedures, but throughout the course of the disease.

5. Cancer Cachexia

Evidence for altered fatty acid metabolism in cancer is supported by plasma and immune cell compositions in the subjects from Chapters VIII (fish oil) and VII (HDCT). A critical issue that needs to be addressed is the severe reduction of PL in the plasma of patients with cancer cacheixa. A prerequisite to observing beneficial effects of nutritional supplementation is the capacity of the patient group of interest to absorb and metabolize the nutrients. In the case of weight losing cancer patients, the ability to incorporate fatty acids into cells of the immune system was not demonstrated. The incorporation of these fats into immune lipids has been postulated as one mechanism for the effect of dietary n-3 supplementation (Robinson,2000). Reduced circulating levels of fatty acids likely contribute to the inability to incorporate supplemental fatty acids into cell membranes. Therefore, attempts to improve immunological parameters based on the beneficial effects of membrane fatty acids may be precluded by biochemical and metabolic abnormalities in the patient group of interest. Correction of the malnourished state may be helpful to further demonstrate beneficial effects of fish oils on attenuating cachexic responses. The effects of the acute phase response, proteins, cytokines and other mediators on essential fatty acid metabolism is an area that requires further investigation.

Multiple treatment strategies currently used to treat cancer patients would be expected to diminish nutritive and immunological status. However, no formal dietary recommendations exist for this patient group. The findings that 14 days of supplementation with a nutrient with few side effects can modulate immune functions (CD8 cells) thought to be important in cancer defense should provide a strong rationale for studying the efficacy of earlier and more aggressive intervention in this group of patients, certainly before they are deemed "palliative". The role of the CD8 subset of lymphocytes in anti-tumor defenses and the subsequent improvements in their function observed with dietary fish oils provides a direction for future research studies.

C. Summary and Conclusion

In conclusion, the subjects investigated in each of these studies represent unique patient groups where nutritional intervention to modulate lipid and immune function using appropriate nutrients is warranted but currently, not well-defined. An understanding of the immune changes that occur following burn injury has advanced considerably in the past decade and nutritional support has become appreciated as an integral part of therapy in a number of other conditions. Although the issue of fatty acid requirements for burn patients remains to be debated, this research offers a significant contribution to the understanding of essential fatty acid metabolism in this group of patients. No formal dietary recommendations exist for cancer patients under palliative care or those undergoing various cancer treatments. It is clear that abnormalities in essential fatty metabolism exist in each of these conditions and are reflected in immune cell membranes. Immunological functions explored in these studies provide evidence for altered immune responses that may relate to the PL composition of immune cell membranes. More research regarding the involvement of fatty acids in the induction of key effector functions of immune cells is needed. These investigations offer the promise of deciphering the complex relationships that exist between dietary fat and immune function in both health and disease.

D. References

Alverdy J, Chi HS, Sheldon GF. The effect of parenteral nutrition on gastrointestinal immunity. The importance of enteral stimulation. Ann Surg 202: 681-684, 1985.

Bragg LE, Thompson JS, Rikkers LF. Influence of nutrient delivery on gut structure and function. Nutrition 7: 237-243, 1991.

Burr GO. and Burr MM. A new deficiency disease produced by the rigid exclusion of fat from the diet. J Biol Chem 82:345-367, 1930

Caughey GE, Mantzioris E, Gibson RA et al. The effect on human tumor necrosis factor alpha and interleukin 1 beta production of diets enriched in n-3 fatty acids from vegetable oil or fish oil. Am J Clin Nutr 63: 116-122, 1996.

Chapkin RS, Ziboh VA, Marcelo CL et al. Metabolism of essential fatty acids by human epidermal enzyme preparations: evidence of chain elongation. Journal of Lipid Research 27: 945-954, 1986.

Clandinin MT, Cheema S, Field CJ et al. Dietary fat: exogenous determination of membrane structure and cell function. FASEB J 5: 2761-2769, 1991.

Cockcroft S and Stutchfield J. The receptors for ATP and fMetLeuPhe are independently coupled to phospholipases C and A2 via G-protein(s). Relationship between phospholipase C and A2 activation and exocytosis in HL60 cells and human neutrophils. Biochem J 263: 715-723, 1989.

Endres S. N-3 polyunsaturated fatty acids and human cytokine synthesis. Lipids 31:S239-S242, 1996

Field CJ, Toyomizu M, Clandinin MT. Relationship between dietary fat, adipocyte membrane composition and insulin binding in the rat. J Nutr 119: 1483-1489, 1989.

Fujishima S and Aikawa N. Neutrophil-mediated tissue injury and its modulation. Int Care Med 21: 277-285, 1995.

Garrel DR, Razi M, Lariviere F et al. Improved clinical status and length of care with low-fat nutrition support in burn patients. JPEN J Parenter Enteral Nutr 19: 482-491, 1995.

Gottschlich MM, Jenkins M, Warden GD et al. Differential effects of three enteral dietary regimens on selected outcome variables in burn patients. JPEN J Parenter Enteral Nutr 14: 225-236, 1990.

Hansbrough JF. Enteral nutritional support in burn patients. Gastro Endosc Clin Nor Am 8: 645-667, 1998.

Hansbrough JF, Wikstrom T, Braide M et al. Neutrophil activation and tissue neutrophil sequestration in a rat model of thermal injury. J Surg Res 61: 17-22, 1996.

Harris RL, Frenkel RA, Cottam GL et al. Lipid mobilization and metabolism after thermal trauma. J Trauma 22: 194-198, 1982.

Hollander D and Tarnawski A. Is there a role for dietary essential fatty acids in gastroduodenal mucosal protection?. J Clin Gastro 13 Suppl 1: S72-S74, 1991.

Hosoda N, Nishi M, Nakagawa M et al. Structural and functional alterations in the gut of parenterally or enterally fed rats. J Surg Res 47: 129-133, 1989.

Hubbard NE, Lim D, Somers SD et al. Effects of in vitro exposure to arachidonic acid on TNF-alpha production by murine peritoneal macrophages. J Leukoc Biol 54: 105-110, 1993.

Kudsk KA, Croce MA, Fabian TC et al. Enteral versus parenteral feeding. Effects on septic morbidity after blunt and penetrating abdominal trauma. Ann Surg 215: 503-11; discussion 511-3, 1992.

Lyons A, Kelly JL, Rodrick ML et al. Major injury induces increased production of interleukin-10 by cells of the immune system with a negative impact on resistance to infection. Ann Surg 226: 450-468, 1997.

Maejima K, Deitch E, Berg R. Promotion by burn stress of the translocation of bacteria from the gastrointestinal tracts of mice. Arch Surg 119: 166-172, 1984.

Mayer AM, Brenic S, Glaser KB. Pharmacological targeting of signaling pathways in protein kinase C-stimulated superoxide generation in neutrophil-like HL- 60 cells: effect of phorbol ester, arachidonic acid and inhibitors of kinase(s), phosphatase(s) and phospholipase A2. J Pharm Exp Ther 279: 633-644, 1996.

McGeer AJ, Detsky AS, O'Rourke K. Parenteral nutrition in cancer patients undergoing chemotherapy: a meta- analysis. Nutrition 6: 233-240, 1990.

Meydani SN. Modulation of cytokine production by dietary polyunsaturated fatty acids. Proc Soc Exp Biol Med 200: 189-193, 1992.

Minard G and Kudsk KA. Effect of route of feeding on the incidence of septic complications in critically ill patients. Semin Respir Infect 9: 228-231, 1994.

Moore FA, Feliciano DV, Andrassy RJ et al. Early enteral feeding, compared with parenteral, reduces postoperative septic complications. The results of a meta-analysis. Ann Surg 216: 172-183, 1992.

Mosmann TR, Li L, Sad S. Functions of CD8 T-cell subsets secreting different cytokine patterns. Semin Immunol 9: 87-92, 1997.

O'Sullivan ST, Lederer JA, Horgan AF et al. Major injury leads to predominance of the T helper-2 lymphocyte phenotype and diminished interleukin-12 production associated with decreased resistance to infection. Ann Surg 222: 482-492, 1995.

Pratt VC, Johnson IR, Field CJ. Glutamine and Arginine: Immunonutrients for improved health. Med Sci Sports Exerc 32: S377-S388, 2000.

Robinson LE. In vivo and In vitro Studies of Anticancer Defense: Diet, Exercise and Immunity. 223-229, 2000. 9-1-2000.

Stanford JL, King I, Kristal AR. Long-term storage of red blood cells and correlations between red cell and dietary fatty acids: results from a pilot study. Nutr Cancer 16: 183-188, 1991.

Tao P and Wilson DE. Effects of prostaglandin E2, 16,16-dimethyl prostaglandin E2 and a prostaglandin endoperoxide analogue (U-46619) on gastric secretory volume, [H+] and mucus synthesis and secretion in the rat. Prostaglandins 28: 353-365, 1984.

Thomson AB, Keelan M, Garg M et al. Dietary effects of omega 3-fatty acids on intestinal transport function. Can J Physiol Pharmacol 66: 985-992, 1988.

Wilson DE. Role of prostaglandins in gastroduodenal mucosal protection. J Clin Gastro 13 Suppl 1: S65-S71, 1991.

Wilson DE, Quertermus J, Raiser M et al. Inhibition of stimulated gastric secretion by an orally administered prostaglandin capsule. A study in normal men. Ann Intern Med 84: 688-691, 1976.

Yaqoob P, Newsholme EA, Calder PC. Fatty acid oxidation by lymphocytes. Biochem Soc Trans 22: 116S, 1994.

Ziboh VA. Essential fatty acids/eicosanoid biosynthesis in the skin: biological significance. Proceedings of the Society for Experimental Biology & Medicine 205: 1-11, 1994.

Ziboh VA, Miller CC, Cho Y. Metabolism of polyunsaturated fatty acids by skin epidermal enzymes: generation of antiinflammatory and antiproliferative metabolites. American Journal of Clinical Nutrition 71: 361S-366S, 2000.

Appendix III.AP1

Fatty Acid	t1	t2	t3	t4	t5
			(% fatty acids)		
14:0	0.9 ± 0.2	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.8 ± 0.2
16:0	31.2 ± 1.5	32.9 ± 1.3	32.3 ± 2.0	27.6 ± 1.3	30.8 ± 0.7
16:1(7)	0.5 ± 0.1^{a}	0.3 ± 0.1^{b}	0.4 ± 0.1^{b}	0.5 ± 0.1^{ab}	0.3 ± 0.1^{b}
16:1(5)	0.8 ± 0.2^{a}	0.5 ± 0.1^{b}	0.6 ± 0.2^{b}	0.6 ± 0.1b	0.5 ± 0.1^{b}
17:0	0.7 ± 0.3^{a}	0.2 ± 0.1^{b}	0.2 ± 0.1^{b}	0.6 ± 0.1^{ab}	0.6 ± 0.1^{ab}
18:0	11.5 ± 1.1	11.8 ± 1.0	11.7 ± 0.9	12.4 ± 1.1	11.5 ± 0.5
18:1(9)	30.6 ± 1.2^{a}	30.7 ± 1.0^{a}	31.4 ± 0.7^{a}	25.6 ± 0.5^{b}	23.6 ± 1.6^{b}
18:1(7)	2.5 ± 0.4^{a}	2.0 ± 0.6^{ab}	1.3 ± 0.5^{b}	2.7 ± 0.3^{ab}	2.1 ± 0.4^{b}
18:2(6)	9.1 ± 1.3	10.7 ± 1.7	9.4 ± 2.0	12.0 ± 2.9	9.5 ± 1.8
18:3(6)	0.3 ± 0.2	0.1 ± 0.1	0.1 ± 0.0	0.3 ± 0.1	nil*
19:0	0.4 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.0	0.2 ± 0.1
18:3(3)	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	0.6 ± 0.3
18:4(3)	0.1 ± 0.0	0.1 ± 0.0	0.4 ± 0.2	0.1 ± 0.0	0.1 ± 0.0
20:0	0.5 ± 0.2^{ab}	0.9 ± 0.3^{a}	0.1 ± 0.1^{b}	0.3 ± 0.1^{ab}	0.3 ± 0.1^{ab}
20:1(9)	0.1 ± 0.0	nil*	0.3 ± 0.2	0.2 ± 0.0	0.2 ± 0.1
20:1(7)	1.2 ± 0.3	1.1 ± 0.2	0.9 ± 0.2	1.2 ± 0.1	1.1 ± 0.1
20:2(6)	0.7 ± 0.3	0.9 ± 0.3	0.8 ± 0.2	0.9 ± 0.2	0.6 ± 0.2
20:3(9)	0.3 ± 0.1^{a}	0.2 ± 0.1^{a}	0.3 ± 0.2^{a}	1.1 ± 0.2^{b}	0.5 ± 0.2^{a}
20:3(6)	0.7 ± 0.1^{a}	1.0 ± 0.3^{ab}	1.1 ± 0.2^{ab}	1.7 ± 0.3^{b}	1.5 ± 0.1b
20:4(6)	3.2 ± 0.4^{a}	3.5 ± 0.4^{a}	4.8 ± 0.5^{b}	5.6 ± 0.7^{bc}	6.6 ± 0.6^{c}
20:5(3)	0.7 ± 0.2	0.8 ± 0.3	0.8 ± 0.6	0.5 ± 0.1	1.0 ± 0.6
22:0	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.3 ± 0.1
22:3(6)	0.1 ± 0.1	nil*	nil*	nil*	nil*
22:4(6)	0.3 ± 0.1^{a}	0.4 ± 0.2^{ab}	0.4 ± 0.2^{ab}	1.0 ± 0.1^{b}	0.6 ± 0.2^{ab}
22:5(6)	0.1 ± 0.0	nil*	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.0
22:5(3)	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.7 ± 0.2	0.4 ± 0.1
24:0	0.1 ± 0.1	nil*	0.1 ± 0.1	0.3 ± 0.1	0.1 ± 0.1
22:6(3)	0.4 ± 0.1	0.4 ± 0.1	0.7 ± 0.3	0.8 ± 0.2	0.6 ± 0.1
24:1(9)	0.1 ± 0.1^{ab}	0.1 ± 0.03^{a}	0.1 ± 0.03^{a}	0.4 ± 0.2^{b}	0.3 ± 0.2^{ab}
Σn-6	14 ± 1	17 ± 1	17 ± 1	14 ± 2	14 ± 2
Σn-3	2 ± 0.3^{a}	2 ± 0.3^{a}	3 ± 0.4^{ab}	3 ± 0.4^{ab}	3 ± 0.4^{b}
ΣPUFA	17 ± 1^{a}	18 ± 1^{a}	19 ± 1^{a}	23 ± 1^{b}	22 ± 1^{b}
ΣSFA	48 ± 1	48 ± 1	49 ± 2	47 ± 2	48 ± 2
ΣMUFA	35 ± 1^{a}	25 ± 1^{a}	34 ± 1^{a}	30 ± 1^{b}	28 ± 1^{b}

*nil=<0.04%

TABLE III.AP1: Fatty acid composition of the PC fraction of neutrophils isolated at specific times during recovery from burn injury was determined by gas chromatography analysis. Data are expressed as means ± SEM (n ≤10). Significant differences during recovery were determined using a repeated measures ANOVA and values within a row not sharing common superscripts are significantly ($p<0.05$) different.

Appendix III.AP2

Fatty Acid	t1	t2	t3	t4	t5
			(% fatty acids)		
14:0	1.6 ± 0.4	1.3 ± 0.3	1.4 ± 0.2	1.1 ± 0.4	1.3 ± 0.5
14:1(9)	0.1 ± 0.04^{ab}	nil^{a*}	0.2 ± 0.1^{b}	0.2 ± 0.1^{ab}	0.3 ± 0.2^{b}
16:0	24.1 ± 2.9^{a}	20.6 ± 4.0^{a}	22.3 ± 2.7^{a}	18.3 ± 3.8^{b}	17.0 ± 5.2^{ab}
16:1(7)	0.6 ± 0.2	0.4 ± 0.2	0.5 ± 0.3	0.6 ± 0.2	0.2 ± 0.2
16:1(5)	0.2 ± 0.1	0.1 ± 0.1	0.4 ± 0.2	0.4 ± 0.1	0.3 ± 0.2
17:0	0.5 ± 0.2	0.5 ± 0.3	0.4 ± 0.2	0.5 ± 0.2	0.6 ± 0.4
18:0	27.7 ± 1.6	28.4 ± 1.5	29.5 ± 1.9	27.1 ± 1.3	29.4 ± 1.7
18:1(9)	11.4 ± 0.8^{a}	11.6 ± 1.5^{ab}	9.7 ± 0.8^{ab}	8.6 ± 1.4^{ab}	7.0 ± 0.9^{b}
18:1(7)	1.2 ± 0.3	0.8 ± 0.4	1.2 ± 0.4	1.4 ± 0.2	1.2 ± 0.4
18:2(6)	5.3 ± 0.5	5.0 ± 1.1	5.1 ± 0.7	5.8 ± 1.0	4.2 ± 0.7
18:3(6)	0.2 ± 0.1	0.3 ± 0.3	0.2 ± 0.1	0.7 ± 0.7	0.2 ± 0.1
19:0	1.6 ± 1.0	0.4 ± 0.3	1.4 ± 0.7	0.6 ± 0.3	1.5 ± 0.5
18:3(3)	0.3 ± 0.2	0.4 ± 0.3	0.5 ± 0.4	0.4 ± 0.2	0.4 ± 0.3
18:4(3)	nil^{a}	0.1 ± 0.1^{ab}	0.2 ± 0.1^{ab}	0.1 ± 0.1^{ab}	0.3 ± 0.2^{b}
20:0	1.2 ± 1.0	0.4 ± 0.3	0.5 ± 0.2	0.6 ± 0.3	0.2 ± 0.1
20:1(9)	0.4 ± 0.3	0.6 ± 0.4	0.4 ± 0.2	0.6 ± 0.1	0.8 ± 0.1
20:1(7)	1.9 ± 0.5	1.8 ± 0.6	1.2 ± 0.4	0.6 ± 0.1	0.9 ± 0.4
20:2(6)	0.8 ± 0.4	0.4 ± 0.2	0.5 ± 0.3	0.9 ± 0.3	0.8 ± 0.2
20:3(9)	0.3 ± 0.1^{a}	0.3 ± 0.2^{a}	0.5 ± 0.2^{a}	1.8 ± 1.0^{b}	0.6 ± 0.3^{a}
20:3(6)	1.7 ± 0.3	3.0 ± 1.0	3.0 ± 0.6	3.6 ± 0.8	3.0 ± 0.7
20:4(6)	8.0 ± 1.2^{a}	10.7 ± 1.7^{ab}	11.1 ± 1.4^{ab}	11.6 ± 2.0^{ab}	17.5 ± 3.9^{b}
20:5(3)	2.7 ± 0.9	1.8 ± 0.8	1.1 ± 0.5	1.3 ± 1.1	0.7 ± 0.6
22:0	0.6 ± 0.4	0.2 ± 0.1	0.7 ± 0.2	1.2 ± 0.6	0.3 ± 0.2
22:1(9)	0.2 ± 0.2	0.7 ± 0.4	0.4 ± 0.1	0.7 ± 0.3	1.0 ± 0.5
22:3(6)	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	1.2 ± 1.2	nil*
22:1(7)	0.1 ± 0.1	0.2 ± 0.2	0.1 ± 0.1	nil*	0.6 ± 0.4
22:4(6)	0.4 ± 0.2	0.6 ± 0.3	0.8 ± 0.2	0.9 ± 0.3	0.9 ± 0.3
22:5(6)	0.6 ± 0.2	0.4 ± 0.3	0.7 ± 0.4	0.1 ± 0.1	0.3 ± 0.3
22:5(3)	0.2 ± 0.1	0.5 ± 0.3	0.2 ± 0.1	0.2 ± 0.1	0.4 ± 0.2
24:0	0.5 ± 0.3	0.8 ± 0.6	0.8 ± 0.5	1.2 ± 0.7	1.1 ± 0.7
22:6(3)	0.2 ± 0.1^{a}	0.2 ± 0.1^{a}	0.5 ± 0.2^{ab}	1.0 ± 0.5^{b}	0.8 ± 0.3^{ab}
24:1(9)	2.2 ± 1.6	3.9 ± 2.8	2.5 ± 1.5	3.5 ± 2.2	4.4 ± 2.6
Σn-6	17 ± 2^{a}	21 ± 2^{ab}	19 ± 2^{ab}	21 ± 3^{ab}	25 ± 3^{b}
Σn-3	3 ± 1	3 ± 1	3 ± 1	5 ± 1	4 ± 1
ΣPUFA	22 ± 2	24 ± 2	22 ± 3	26 ± 4	29 ± 3
ΣSFA	61 ± 1^{a}	56 ± 2^{b}	62 ± 2^{a}	51 ± 3^{b}	59 ± 2^{ac}
ΣMUFA	17 ± 1	18 ± 1	17 ± 1	19 ± 2	16 ± 2

*nil=<0.04%

TABLE III.AP2: Fatty acid composition of the PI fraction of neutrophils isolated at specific times during recovery from burn injury was determined by gas chromatography analysis. Data are expressed as means ± SEM (n ≤10). Significant differences during recovery were determined using a repeated measures ANOVA and values within a row not sharing common superscripts are significantly ($p<0.05$) different.

Appendix III.AP3

Fatty Acid	t1	t2	t3	t4	t5
			(% fatty acids)		
14:0	1.2 ± 0.3	1.0 ± 0.3	0.6 ± 0.1	1.5 ± 0.1	1.2 ± 0.4
14:1(9)	0.1 ± 0.1	nil*	nil*	0.1 ± 0.1	nil*
15:0	0.9 ± 0.2	0.8 ± 0.3	0.7 ± 0.2	0.7 ± 0.1	0.7 ± 0.2
16:0	14.5 ± 2.7	13.8 ± 1.8	11.4 ± 1.8	15.1 ± 1.9	11.8 ± 2.1
16:1(7)	0.5 ± 0.2^{a}	0.3 ± 0.1^{ab}	0.1 ± 0.1^{b}	0.8 ± 0.4^{ab}	0.4 ± 0.1^{ab}
16:1(5)	0.2 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	0.4 ± 0.1	0.5 ± 0.1
17:0	0.5 ± 0.3	1.2 ± 0.5	1.4 ± 0.7	1.0 ± 0.3	1.2 ± 0.9
18:0	31.6 ± 2.6	32.6 ± 1.8	36.1 ± 2.2	33.8 ± 2.3	32.6 ± 1.8
18:1(9)	26.4 ± 3.7	26.2 ± 2.5	29.2 ± 2.1	23.8 ± 2.9	23.8 ± 2.9
18:1(7)	1.5 ± 1.1	0.4 ± 0.2	0.3 ± 0.1	1.1 ± 0.4	0.8 ± 0.1
18:2(6)	4.0 ± 0.5	3.9 ± 0.5	4.2 ± 0.6	4.2 ± 0.7	2.5 ± 0.3
18:3(6)	0.2 ± 0.2^{a}	1.0 ± 0.7^{b}	0.1 ± 0.1^{ab}	0.4 ± 0.3^{ab}	0.1 ± 0.1^{ab}
19:0	1.4 ± 1.3	0.7 ± 0.5	0.9 ± 0.6	0.7 ± 0.4	1.4 ± 0.5
18:3(3)	0.3 ± 0.2^{ab}	0.6 ± 0.3^{a}	0.1 ± 0.1^{b}	0.2 ± 0.2^{ab}	0.6 ± 0.5^{ab}
18:4(3)	0.2 ± 0.1^{a}	0.9 ± 0.6^{b}	nil^{a*}	nil^{a*}	0.1 ± 0.1^{a}
20:0	0.2 ± 0.1^{a}	0.8 ± 0.3^{b}	0.4 ± 0.2^{a}	0.6 ± 0.2^{ab}	1.0 ± 0.4^{ab}
20:1(9)	0.2 ± 0.1^{ab}	0.5 ± 0.2^{a}	0.1 ± 0.04^{b}	0.3 ± 0.2^{ab}	0.5 ± 0.3^{ab}
20:1(7)	3.1 ± 1.1^{a}	1.4 ± 0.4^{b}	1.6 ± 0.8^{ab}	0.4 ± 0.1^{ab}	0.5 ± 0.1^{ab}
20:2(6)	0.4 ± 0.2	1.4 ± 1.3	0.3 ± 0.2	1.5 ± 1.1	2.4 ± 2.1
20:3(9)	0.3 ± 0.1	0.5 ± 0.3	0.2 ± 0.1	0.7 ± 0.2	0.4 ± 0.2
20:3(6)	1.5 ± 0.4	0.8 ± 0.3	1.5 ± 0.3	1.8 ± 0.2	1.9 ± 0.4
20:4(6)	4.6 ± 0.5^{ab}	3.8 ± 0.5^{a}	4.4 ± 0.6^{ab}	6.2 ± 0.8^{b}	9.6 ± 0.8^{c}
20:5(3)	1.4 ± 0.4	2.4± 1.2	1.2 ± 0.6	0.4 ± 0.3	0.2 ± 0.1
22:0	0.6 ± 0.3	0.2 ± 0.1	0.3 ± 0.2	0.3 ± 0.3	0.2 ± 0.2
22:1(9)	0.2 ± 0.1^{a}	0.3 ± 0.2^{a}	0.2 ± 0.1^{a}	0.4 ± 0.2^{ab}	0.6 ± 0.3^{b}
22:3(6)	0.1 ± 0.1	0.2 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.2
22:1(7)	0.1 ± 0.1	nil*	0.1 ± 0.1	0.2 ± 0.2	nil*
22:4(6)	0.8 ± 0.5	0.3 ± 0.2	0.6 ± 0.2	0.9 ± 0.2	1.4 ± 0.3
22:5(6)	0.3 ± 0.1	0.5 ± 0.5	0.7 ± 0.5	nil*	0.3 ± 0.1
22:5(3)	0.2 ± 0.1	0.9 ± 0.4	0.8 ± 0.4	0.5 ± 0.2	1.4 ± 0.7
22:6(3)	0.5 ± 0.3	0.2 ± 0.1	0.4 ± 0.3	0.6 ± 0.1	0.9 ± 0.3
24:0	0.3 ± 0.2	0.2 ± 0.1	0.3 ± 0.2	0.5 ± 0.3	0.4 ± 0.3
22:6(3)	0.5 ± 0.3	0.2 ± 0.1	0.4 ± 0.3	0.6 ± 0.1	0.9 ± 0.3
24:1(9)	0.7 ± 0.3	0.5 ± 0.3	0.8 ± 0.4	1.0 ± 0.6	0.6 ± 0.4
Σn-6	12 ± 1^{a}	11 ± 1^{a}	12 ± 1^{a}	14 ± 1^{ab}	17 ± 1^{b}
Σn-3	2 ± 1	5 ± 1	3 ± 1	3 ± 2	4 ± 2
ΣPUFA	15 ± 1^{a}	17 ± 1^{ab}	14 ± 2^{a}	17 ± 2^{ab}	21 ± 2^{b}
ΣSFA	33 ± 2	32 ± 2	32 ± 2	29 ± 3	32 ± 3
ΣMUFA	51 ± 2	51 ± 2	52 ± 2	51 ± 3	48 ± 3

*nil=<0.04%

TABLE III.AP3: Fatty acid composition of the PS fraction of neutrophils isolated at specific times during recovery from burn injury was determined by gas chromatography analysis. Data are expressed as means ± SEM (n ≤10). Significant differences during recovery were determined using a repeated measures ANOVA and values within a row not sharing common superscripts are significantly (p<0.05) different.

Appendix III.AP4

Fattv Acid	t1	t2	t3	t4	t5
			(% fatty acids)		
14:0	0.8 ± 0.3	1.2 ± 0.3	1.0 ± 0.3	1.0 ± 0.4	1.0 ± 0.4
14:1(9)	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.1
15:0	1.0 ± 0.4	1.1 ± 0.6	0.6 ± 0.2	0.4 ± 0.1	0.4 ± 0.2
16:0	12.1 ± 1.6	12.6 ± 1.9	14.1 ± 2.1	13.0 ± 2.4	11.9 ± 3.3
16:1(7)	0.8 ± 0.2	0.6 ± 0.2	0.4 ± 0.2	0.5 ± 0.3	0.5 ± 0.3
16:1(5)	0.5 ± 0.2^{a}	0.4 ± 0.3^{a}	0.4 ± 0.2^{ab}	0.3 ± 0.1^{b}	0.5 ± 0.3^{ab}
17:0	2.9 ± 1.1^{a}	1.3 ± 0.5^{b}	1.5 ± 0.6^{b}	0.8 ± 0.1^{ab}	0.9 ± 0.3^{b}
18:0	16.5 ± 1.0	15.9 ± 1.0	17.0 ± 1.2	17.1 ± 1.1	17.0 ± 1.9
18:1(9)	24.0 ± 2.7	27.1 ± 2.2	23.2 ± 1.6	19.6 ± 1.5	19.7 ± 2.6
18:1(7)	0.8 ± 0.2	0.8 ± 0.3	0.9 ± 0.3	1.3 ± 0.5	1.0 ± 0.4
18:2(6)	8.8 ± 1.7	9.3 ± 0.6	8.3 ± 1.0	10.2 ± 1.1	6.9 ± 0.6
18:3(6)	0.1 ± 0.1	0.3 ± 0.2	0.1 ± 0.1	0.2 ± 0.2	0.3 ± 0.2
19:0	0.3 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.5 ± 0.3	0.5 ± 0.2
18:3(3)	0.4 ± 0.1^{a}	1.3 ± 0.3^{b}	1.0 ± 0.4^{b}	1.1 ± 0.2^{ab}	1.0 ± 0.3^{ab}
18:4(3)	0.5 ± 0.2	0.2 ± 0.1	0.5 ± 0.2	0.5 ± 0.1	0.4 ± 0.2
20:0	0.3 ± 0.1	0.4 ± 0.2	0.3 ± 0.1	0.4 ± 0.2	0.3 ± 0.1
20:1(9)	2.3 ± 2.1	0.5 ± 0.4	0.1 ± 0.0	0.2 ± 0.1	0.3 ± 0.2
20:1(7)	1.3 ± 0.5	0.9 ± 0.3	2.2 ± 1.3	0.6 ± 0.1	0.5 ± 0.2
20:2(6)	0.5 ± 0.1	0.7 ± 0.3	0.3 ± 0.2	0.8 ± 0.1	0.8 ± 0.4
20:3(9)	0.3 ± 0.1^{a}	0.2 ± 0.1^{a}	0.5 ± 0.2^{ab}	0.9 ± 0.2^{b}	0.4 ± 0.2^{ab}
20:3(6)	1.2 ± 0.2	2.2 ± 0.3	2.2 ± 0.5	2.3 ± 0.3	1.7 ± 0.4
20:4(6)	13.6 ± 1.4^{a}	14.5 ± 2.2^{ab}	15.4 ± 1.7^{ab}	16.6 ± 1.8^{ab}	20.6 ± 3.9^{ab}
20:5(3)	1.8 ± 0.7	1.2 ± 0.4	1.0 ± 0.4	0.5 ± 0.2	0.8 ± 0.4
22:0	1.2 ± 0.7^{a}	0.1 ± 0.1^{ab}	0.2 ± 0.1^{ab}	0.3 ± 0.2^{ab}	0.1 ± 0.1^{b}
22:1(9)	0.1 ± 0.0	0.3 ± 0.2	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.1
22:3(6)	0.1 ± 0.1	0.1 ± 0.1	nil*	0.1 ± 0.1	0.1 ± 0.1
22:1(7)	0.1 ± 0.1	nil*	0.1 ± 0.1	0.2 ± 0.2	nil*
22:4(6)	2.2 ± 1.0	2.7 ± 1.3	2.4 ± 0.9	4.2 ± 0.4	5.7 ± 1.6
22:5(6)	0.3 ± 0.2	0.4 ± 0.1	0.5 ± 0.2	0.5 ± 0.1	0.8 ± 0.4
22:5(3)	1.9 ± 0.4	1.7 ± 0.7	1.4 ± 0.5	2.2 ± 0.4	2.0 ± 0.4
24:0	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	0.4 ± 0.3	0.4 ± 0.2
22:6(3)	1.4 ± 0.4	1.3 ± 0.7	1.9 ± 0.7	2.0 ± 0.5	2.0 ± 0.6
24:1(9)	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.4 ± 0.3	0.3 ± 0.2
Σn-6	26 ± 2	27 ± 2	27 ± 2	29 ± 3	33 ± 3
Σn-3	6 ± 1	7 ± 1	6 ± 1	6 ± 1	6 ± 1
ΣPUFA	30 ± 27^{a}	32 ± 2^{ab}	37 ± 2^{b}	37 ± 3^{bc}	44 ± 2^{c}
ΣSFA	37 ± 2	35 ± 2	39 ± 2	38 ± 3	31 ± 2
ΣMUFA	29 ± 1^{a}	29 ± 1^{a}	26 ± 1^{b}	26 ± 1^{b}	25 ± 1^{b}

*nil=<0.04%

TABLE III.AP4: Fatty acid composition of the PE fraction of neutrophils isolated at specific times during recovery from burn injury was determined by gas chromatography analysis. Data are expressed as means ± SEM (n ≤10). Significant differences during recovery were determined using a repeated measures ANOVA and values within a row not sharing common superscripts are significantly ($p<0.05$) different.

Appendix III.AP5

Fatty Acid	*PS*	*PS*	*PI*	*PC*
	(% of total fatty acids)			
C 12:0	0.3 ± 0.2	0.5 ± 0.2	1.2 ± 0.5	nil*
C 14:0	3.3 ± 0.9	1.6 ± 0.6	2.0 ± 0.6	0.6 ± 0.2
C 14:1(9)	nil*	nil*	nil*	0.1 ± 0.1
C 15:0	1.0 ± 0.2	2.8 ± 0.8	0.5 ± 0.2	0.1 ± 0.1
C 16:0	28.5 ± 4.9	14.8 ± 5.0	17.6 ± 4.2	32.5 ± 1.4
C 16:1(7)	0.9 ± 0.3	0.4 ± 0.1	4.7 ± 4.3	0.2 ± 0.2
C 16:1(5)	0.9 ± 0.4	0.7 ± 0.3	1.0 ± 0.7	0.4 ± 0.2
C 17:0	1.7 ± 0.3	1.9 ± 1.1	0.4 ± 0.4	0.2 ± 0.1
C 18:0	28.5 ± 4.7	17.2 ± 2.0	25.2 ± 4.5	11.9 ± 0.8
C 18:1(9)	18.1 ± 1.8	16.5 ± 2.9	6.9 ± 1.4	24.6 ± 0.4
C 18:1(7)	0.4 ± 0.4	1.4 ± 0.4	7.2 ± 5.2	4.0 ± 0.9
C 18:2(6)	3.1 ± 0.6	5.0 ± 0.9	2.7 ± 0.9	9.6 ± 0.6
C 19:0	0.4 ± 0.4	0.8 ± 0.2	1.0 ± 0.6	nil*
C 18:3(3)	1.2 ± 0.3	0.4 ± 0.2	1.8 ± 1.4	nil*
C 18:4(3)	nil*	0.2 ± 0.1	0.1 ± 0.1	nil*
C 20:0 ±	0.2 ± 0.2	0.4 ± 0.1	1.2 ± 0.8	0.7 ± 0.3
C 20:1(9)	0.9 ± 0.1	0.3 ± 0.1	0.4 ± 0.2	nil*
C 20:1(7)	0.2 ± 0.1	0.2 ± 0.1	0.4 ± 0.2	0.3 ± 0.3
C 20:2(6)	1.7 ± 0.4	0.5 ± 0.2	0.5 ± 0.3	0.5 ± 0.3
C 20:3(9)	0.4 ± 0.2	0.3 ± 0.2	0.5 ± 0.2	0.3 ± 0.3
C 20:3(6)	0.6 ± 0.2	5.4 ± 4.3	2.3 ± 0.7	0.9 ± 0.3
C 20:4(6)	5.4 ± 2.2	16.3 ± 5.0	12.4 ± 3.6	7.7 ± 0.8
C 20:5(3)	0.2 ± 0.2	0.4 ± 0.1	0.7 ± 0.3	1.9 ± 0.9
C 22:0 ±	0.5 ± 0.2	0.3 ± 0.2	0.7 ± 0.2	0.1 ± 0.1
C 22:1(9)	nil*	0.0 ± 0.0	0.3 ± 0.1	nil*
C 22:1(7)	nil*	0.0 ± 0.0	0.2 ± 0.1	nil*
C 22:4(6)	0.4 ± 0.2	2.6 ± 0.6	0.5 ± 0.2	0.5 ± 0.3
C 22:5(6)	nil*	0.2 ± 0.1	3.5 ± 1.9	2.2 ± 1.0
C 22:5(3)	0.2 ± 0.2	1.8 ± 0.4	0.3 ± 0.1	0.2 ± 0.1
C 22:6(3)	0.5 ± 0.3	1.9 ± 0.3	0.8 ± 0.2	0.3 ± 0.2
C 24:1(9)	0.2 ± 0.1	0.1 ± 0.1	0.8 ± 0.5	nil*

*nil=<0.04%

TABLE III.AP5: Fatty acid composition of neutrophils isolated from healthy subjects was determined by gas chromatography analysis. Data are expressed as means ± SEM (n=6).

Appendix IV.AP1

Fatty acid	t1	t2	t3 *(% fatty acids)*	t4	t5
14:0	0.9 ± 0.3	0.7 ± 0.3	0.6 ± 0.1	0.8 ± 0.2	1.3 ± 0.4
14:1(9)	0.2 ± 0.1^{a}	0.1 ± 0.1^{b}	0.0 ± 0.0^{bc}	0.1 ± 0.1^{ac}	0.1 ± 0.1^{ac}
15:0	0.6 ± 0.2	0.4 ± 0.1	0.6 ± 0.2	0.4 ± 0.1	0.5 ± 0.1
16:0	28.4 ± 0.8^{a}	33.6 ± 1.0^{b}	30.7 ± 1.1^{b}	28.2 ± 1.8^{ab}	30.0 ± 1.7^{ab}
16:1(7)	0.5 ± 0.2^{ab}	0.6 ± 0.1^{a}	0.8 ± 0.1^{ab}	0.6 ± 0.2^{ab}	0.3 ± 0.1^{b}
16:1(5)	0.6 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.2
17:0	0.9 ± 0.2^{a}	0.5 ± 0.10^{a}	0.5 ± 0.2^{a}	1.1 ± 0.3^{b}	2.2 ± 0.6^{b}
18:0	15.1 ± 1.1	12.7 ± 1.2	13.1 ± 1.0	13.8 ± 1.1	14.4 ± 0.7
18:1(9)	23.9 ± 1.6^{ab}	24.7 ± 1.8^{a}	24.8 ± 1.2^{a}	22.5 ± 1.3^{ab}	19.6 ± 1.7^{b}
18:1(7)	3.1 ± 0.3	3.2 ± 0.3	3.1 ± 0.2	2.7 ± 0.2	2.4 ± 0.1
18:2(6)	8.3 ± 1.0	7.5 ± 0.6	8.8 ± 1.3	10.7 ± 0.7	8.1 ± 0.9
18:3(6)	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.0 ± 0.0
19:0	0.2 ± 0.1^{a}	0.2 ± 0.1^{ab}	0.1 ± 0.0^{a}	0.7 ± 0.3^{ab}	1.7 ± 1.0^{b}
18:3(3)	0.3 ± 0.1^{a}	0.1 ± 0.1^{b}	0.1 ± 0.1^{b}	0.2 ± 0.1^{ab}	0.1 ± 0.1^{ab}
18:4(3)	0.5 ± 0.2	0.2 ± 0.1	0.4 ± 0.2	0.1 ± 0.0	0.2 ± 0.1
20:0	0.9 ± 0.4^{ab}	0.8 ± 0.2^{ab}	0.6 ± 0.1^{a}	0.6 ± 0.1^{ab}	1.9 ± 1.0^{b}
20:1(9)	0.7 ± 0.2	0.8 ± 0.3	0.2 ± 0.2	0.3 ± 0.1	0.4 ± 0.1
20:1(7)	0.7 ± 0.2	1.0 ± 0.3	0.6 ± 0.2	1.2 ± 0.1	1.0 ± 0.2
20:2(6)	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.2	1.0 ± 0.3	0.6 ± 0.2
20:3(9)	0.5 ± 0.1	0.6 ± 0.2	0.9 ± 0.3	0.7 ± 0.2	0.6 ± 0.1
20:3(6)	1.3 ± 0.2^{a}	1.4 ± 0.2^{ab}	1.0 ± 0.3^{a}	1.8 ± 0.2^{ab}	2.2 ± 0.4^{b}
20:4(6)	6.6 ± 1.0^{a}	6.5 ± 0.1^{a}	7.8 ± 0.8^{ab}	8.4 ± 1.2^{ab}	10.3 ± 1.0^{b}
20:5(3)	0.4 ± 0.1^{a}	0.2 ± 0.1^{b}	0.2 ± 0.1^{b}	0.2 ± 0.1^{ab}	0.3 ± 0.1^{ab}
22:0	0.3 ± 0.1^{a}	0.1 ± 0.0^{b}	0.2 ± 0.1^{ab}	0.3 ± 0.1^{ab}	0.1 ± 0.1^{ab}
22:1(9)	0.1 ± 0.0^{ab}	0.0 ± 0.0^{ab}	0.0 ± 0.0^{a}	0.1 ± 0.0^{ab}	0.5 ± 0.3^{b}
22:1(7)	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0
22:4(6)	0.6 ± 0.1	0.7 ± 0.2	0.5 ± 0.1	0.9 ± 0.1	0.6 ± 0.1
22:5(6)	0.6 ± 0.4	0.1 ± 0.1	0.7 ± 0.5	0.3 ± 0.1	0.1 ± 0.0
22:5(3)	0.8 ± 0.1^{ab}	0.6 ± 0.1^{ab}	0.7 ± 0.1^{ab}	1.0 ± 0.2^{a}	0.5 ± 0.1^{b}
24:0	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.4 ± 0.2	0.1 ± 0.0
22:6(3)	1.5 ± 0.5	0.8 ± 0.1	0.8 ± 0.1	1.1 ± 0.2	0.8 ± 0.1
24:1(9)	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
Σn-6	18 ± 1^{ab}	17 ± 1^{a}	20 ± 1^{b}	21 ± 2^{ab}	20 ± 2^{ab}
Σn-3	4 ± 0.4^{a}	2 ± 0.4^{b}	2 ± 0.5^{b}	3 ± 1^{ab}	2 ± 1^{b}
ΣPUFA	22 ± 1^{ab}	21 ± 1^{a}	24 ± 1^{b}	24 ± 1^{b}	23 ± 1^{ab}
ΣSFA	46 ± 1^{a}	47 ± 1^{a}	46 ± 2^{a}	49 ± 2^{ab}	53 ± 2^{b}
ΣMUFA	31 ± 1^{a}	32 ± 1^{a}	30 ± 1^{ac}	28 ± 1^{bc}	26 ± 1^{b}

TABLE IV.AP1: Fatty acid composition of the PC fraction of lymphocytes at post burn timepoints expressed a percent of total fatty acids. Data are expressed as means ± SEM (n ≤10). Significant differences between post burn timepoints were determined using a repeated measures ANOVA and values within a row not sharing common superscripts are significantly ($p<0.05$) different.

Appendix IV.AP2

Fatty acid	t1	t2	t3	t4	t5
			(% fatty acids)		
14:0	1.1 ± 0.4	0.9 ± 0.3	1.0 ± 0.4	1.9 ± 0.8	1.6 ± 0.5
14:1(9)	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	nil*
15:0	2.1 ± 1.0	1.1 ± 0.3	0.7 ± 0.3	1.7 ± 0.5	0.7 ± 0.9
16:0	16.3 ± 2.9^{a}	10.2 ± 2.3^{b}	12.5 ± 5.1^{ab}	20.8 ± 5.5^{ab}	15.7 ± 4.5^{ab}
16:1(7)	0.4 ± 0.2	0.4 ± 0.1	0.4 ± 0.2	0.7 ± 0.5	0.6 ± 0.2
16:1(5)	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.5 ± 0.2	0.5 ± 0.2
17:0	1.6 ± 0.3^{a}	1.1 ± 0.2^{b}	0.9 ± 0.4^{b}	1.9 ± 0.5^{a}	0.8 ± 0.2^{b}
18:0	30.6 ± 1.1	36.7 ± 1.8	30.8 ± 12.6	27.7 ± 2.7	29.6 ± 3.8
18:1(9)	18.2 ± 1.8^{a}	24.8 ± 1.4^{b}	22.0 ± 9.0^{ab}	16.5 ± 3.2^{ab}	19.1 ± 2.7^{ab}
18:1(7)	0.5 ± 0.2	0.5 ± 0.1	0.7 ± 0.3	1.0 ± 0.8	0.4 ± 0.1
18:2(6)	2.9 ± 0.4	2.1 ± 0.4	2.3 ± 0.9	3.6 ± 0.7	2.6 ± 0.7
18:3(6)	0.3 ± 0.2^{ab}	0.2 ± 0.1^{ab}	1.1 ± 0.4^{a}	0.7 ± 0.4^{ab}	0.1 ± 0.1^{b}
19:0	1.2 ± 0.5	0.7 ± 0.3	0.6 ± 0.2	1.3 ± 0.5	0.8 ± 0.4
18:3(3)	0.5 ± 0.4	0.1 ± 0.1	0.3 ± 0.1	0.8 ± 0.5	0.2 ± 0.1
18:4(3)	1.7 ± 1.2	0.8 ± 0.4	0.3 ± 0.1	0.1 ± 0.1	0.1 ± 1.3
20:0	1.0 ± 0.2	1.6 ± 0.3	1.7 ± 0.7	0.9 ± 0.3	0.6 ± 0.3
20:1(9)	0.7 ± 0.4	0.7 ± 0.1	0.5 ± 0.2	0.1 ± 0.1	1.0 ± 0.3
20:1(7)	1.2 ± 0.4	0.6 ± 0.1	0.9 ± 0.4	0.2 ± 0.1	0.5 ± 0.5
20:2(6)	1.3 ± 1.1	0.6 ± 0.3	0.3 ± 0.1	1.3 ± 0.8	1.2 ± 0.6
20:3(9)	0.4 ± 0.2	0.2 ± 0.1	1.1 ± 0.5	1.0 ± 0.6	0.3 ± 0.1
20:3(6)	1.2 ± 0.2^{a}	1.8 ± 0.3^{b}	2.1 ± 0.9^{b}	1.4 ± 0.7^{ab}	2.3 ± 0.5^{b}
20:4(6)	7.9 ± 1.0^{a}	9.5 ± 1.0^{ab}	11.3 ± 1.2^{b}	12.3 ± 1.6^{b}	17.2 ± 1.4^{c}
20:5(3)	0.4 ± 0.2	0.2 ± 0.1	1.0 ± 0.4	0.7 ± 0.6	0.8 ± 0.6
22:0	0.5 ± 0.2	0.5 ± 0.3	0.5 ± 0.2	0.3 ± 0.3	0.6 ± 0.4
22:1(9)	0.4 ± 0.2	0.3 ± 0.1	0.2 ± 0.1	0.5 ± 0.3	0.67 ± 0.3
22:3(6)	0.1 ± 0.1	0.1 ± 0.1	nil*	0.2 ± 0.2	0.2 ± 0.1
22:1(7)	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.7 ± 0.7	nil*
22:4(6)	0.9 ± 0.2	0.9 ± 0.2	1.2 ± 0.5	0.8 ± 0.4	1.0 ± 0.3
22:5(6)	0.2 ± 0.0	0.1 ± 0.1	0.3 ± 0.1	0.5 ± 0.5	0.2 ± 0.1
22:5(3)	0.7 ± 0.2	0.5 ± 0.1	1.4 ± 0.6	0.3 ± 0.1	0.5 ± 0.1
24:0	0.3 ± 0.2^{ab}	0.2 ± 0.2^{ab}	0.3 ± 0.1^{ab}	0.8 ± 0.5^{a}	0.3 ± 0.1^{b}
22:6(3)	1.5 ± 0.3	1.0 ± 0.1	1.4 ± 0.6	0.6 ± 0.3	1.0 ± 0.2
24:1(9)	0.7 ± 0.2^{a}	0.5 ± 0.3^{ab}	0.2 ± 0.1^{b}	0.7 ± 0.5^{ab}	0.5 ± 0.2^{ab}
Σn-6	15 ± 1^{a}	15 ± 1^{a}	17 ± 2^{a}	20 ± 2^{ab}	23 ± 2^{b}
Σn-3	4 ± 1^{a}	3 ± 1^{b}	3 ± 1^{ab}	3 ± 1^{ab}	5 ± 1^{a}
ΣPUFA	20 ± 2^{a}	19 ± 2^{a}	24 ± 2^{ab}	25 ± 2^{ab}	26 ± 2^{b}
ΣSFA	55 ± 2^{a}	53 ± 2^{ab}	55 ± 2^{a}	55 ± 3^{ab}	48 ± 3^{b}
ΣMUFA	24 ± 2^{ab}	28 ± 2^{a}	26 ± 2^{ab}	21 ± 3^{b}	24 ± 2^{ab}

*nil=<0.04

TABLE IV.AP2: Fatty acid composition of the PS fraction of lymphocytes at post burn timepoints expressed a percent of total fatty acids. Data are expressed as means ± SEM (n ≤10). Significant differences between post burn timepoints were determined using a repeated measures ANOVA and values within a row not sharing common superscripts are significantly ($p<0.05$) different.

Appendix IV.AP3

Fatty Acid	t1	t2	t3	t4	t5
			(% fatty acids)		
14:0	1.6 ± 0.6	1.0 ± 0.2	1.6 ± 0.5	0.8 ± 0.5	2.0 ± 0.6
14:1 (9)	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.2	0.1 ± 0.1	0.5 ± 0.3
15:0	4.0 ± 1.2^{a}	1.7 ± 0.6^{b}	1.2 ± 0.2^{b}	0.7 ± 0.4^{ab}	0.7 ± 0.2^{b}
16:0	16.8 ± 3.1	13.2 ± 2.3	20.3 ± 2.5	14.5 ± 5.9	18.6 ± 5.7
16:1 (7)	0.5 ± 0.3	0.5 ± 0.2	0.5 ± 0.1	0.8 ± 0.5	0.5 ± 0.2
16:1 (5)	0.3 ± 0.1	0.2 ± 0.1	0.4 ± 0.2	0.4 ± 0.2	0.8 ± 0.4
17:0	0.9 ± 0.2	0.5 ± 0.2	0.9 ± 0.4	0.7 ± 0.4	0.5 ± 0.3
18:0	26.1 ± 2.5^{a}	36.2 ± 2.2^{b}	27.8 ± 3.5^{ab}	25.8 ± 2.6^{ab}	29.1 ± 3.4^{ab}
18:1 (9)	7.9 ± 1.2	7.7 ± 0.5	7.1 ± 1.1	8.2 ± 1.9	8.6 ± 2.6
18:1 (7)	1.0 ± 0.2^{ab}	1.6 ± 0.2^{ab}	0.8 ± 0.3^{a}	2.1 ± 0.6^{b}	1.0 ± 0.3^{ab}
18:2 (6)	2.3 ± 0.6	3.0 ± 0.5	3.3 ± 0.5	4.5 ± 1.2	2.6 ± 0.3
18:3 (6)	1.1 ± 0.9	0.1 ± 0.1	0.3 ± 0.2	0.5 ± 0.3	0.4 ± 0.3
19:0	0.9 ± 0.4	0.3 ± 0.1	2.3 ± 1.4	0.8 ± 0.6	1.1 ± 0.4
18:3 (3)	0.3 ± 0.2	0.2 ± 0.1	0.4 ± 0.3	0.6 ± 0.5	0.7 ± 0.5
18:4 (3)	3.1 ± 1.5	1.1 ± 0.6	1.0 ± 0.7	0.6 ± 0.6	0.1 ± 0.1
20:0	1.7 ± 1.8	0.7 ± 0.1	1.9 ± 1.1	4.1 ± 3.5	0.5 ± 0.2
20:1 (9)	0.3 ± 0.1^{a}	0.3 ± 0.2^{a}	nil^{a*}	0.3 ± 0.1^{a}	0.6 ± 0.2^{b}
20:1 (7)	1.1 ± 0.4^{a}	0.9 ± 0.2^{a}	0.8 ± 0.3^{a}	3.1 ± 2.9^{b}	0.6 ± 0.3^{a}
20:2 (6)	0.5 ± 0.2^{a}	0.4 ± 0.2^{a}	0.8 ± 0.6^{a}	2.4 ± 1.7^{b}	0.3 ± 0.2^{a}
20:3 (9)	1.6 ± 1.4	0.3 ± 0.2	0.4 ± 0.3	3.5 ± 2.5	0.5 ± 0.2
20:3 (6)	1.0 ± 0.2	1.8 ± 0.3	1.6 ± 0.5	2.0 ± 0.8	1.1 ± 0.4
20:4 (6)	13.2 ± 2.5^{a}	22.9 ± 3.3^{b}	14.6 ± 3.4^{ab}	15.3 ± 5.6^{ab}	21.5 ± 7.1^{b}
20:5 (3)	2.0 ± 1.3	0.3 ± 0.1	0.3 ± 0.2	0.6 ± 0.4	0.25 ± 0.18
22:0	0.5 ± 0.2^{a}	0.3 ± 0.1^{ab}	0.3 ± 0.2^{ab}	0.6 ± 0.5^{ab}	0.1 ± 0.1^{b}
22:1 (9)	0.2 ± 0.1	0.4 ± 0.1	0.4 ± 0.2	0.6 ± 0.3	0.8 ± 0.4
22:3 (6)	0.1 ± 0.1^{ab}	0.1 ± 0.0^{a}	0.1 ± 0.1^{ab}	0.5 ± 0.5^{b}	nil^{a*}
22:1 (7)	nil*	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.17
22:4 (6)	0.3 ± 0.1	0.6 ± 0.2	0.5 ± 0.3	0.8 ± 0.1	0.5 ± 0.2
22:5 (6)	0.2 ± 0.1	nil*	1.0 ± 0.8	0.3 ± 0.3	0.1 ± 0.1
22:5 (3)	3.1 ± 2.1	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.2	0.1 ± 0.1
24:0	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	1.1 ± 0.7	1.7 ± 1.4
22:6 (3)	0.8 ± 0.3	0.5 ± 0.1	0.4 ± 0.2	0.6 ± 0.4	0.8 ± 0.4
24:1 (9)	1.5 ± 1.1	0.8 ± 0.4	0.7 ± 0.5	1.6 ± 0.9	1.5 ± 0.8
Σn-6	19 ± 3^{a}	31 ± 3^{b}	26 ± 4^{ab}	24 ± 6^{ab}	29 ± 4^{ab}
Σn-3	8 ± 2^{a}	2 ± 2^{b}	2 ± 2^{b}	7 ± 3^{ab}	3 ± 2^{ab}
ΣPUFA	32 ± 3	33 ± 3	29 ± 3	32 ± 5	32 ± 4
ΣSFA	12 ± 1	13 ± 1	12 ± 1	16 ± 2	13 ± 2
ΣMUFA	55 ± 3	52 ± 3	58 ± 4	53 ± 6	54 ± 5

*nil=<0.04

Table IV.AP3: Fatty acid composition of the PI fraction of Lymphocytes at post burn timepoints expressed a percent of total fatty acids. Data are expressed as means ± SEM (n ≤10). Significant differences between post burn timepoints were determined using a repeated measures ANOVA and values within a row not sharing common superscripts are significantly (p<0.05) different.

Appendix IV.AP4

Fatty Acid	t1	t2	t3	t4	t5
			(% fatty acids)		
14:0	1.7 ± 0.4	1.1 ± 0.3	1.2 ± 0.3	0.8 ± 0.5	0.9 ± 0.4
14:1 (9)	0.1 ± 0.1	nil	0.5 ± 0.3	0.1 ± 0.1	0.2 ± 0.1
15:0	5.1 ± 3.0	2.5 ± 1.1	2.0 ± 0.9	0.2 ± 0.1	0.6 ± 0.2
16:0	21.3 ± 3.0^{a}	12.6 ± 1.9^{b}	13.0 ± 1.4^{b}	12.5 ± 5.7^{ab}	16.8 ±
16:1 (7)	0.8 ± 0.3	0.3 ± 0.1	0.4 ± 0.3	0.2 ± 0.1	0.4 ± 0.1
16:1 (5)	1.1 ± 0.3	0.8 ± 0.3	0.5 ± 0.2	0.4 ± 0.1	0.6 ± 0.2
17:0	0.9 ± 0.3	1.1 ± 0.6	2.3 ± 1.6	0.3 ± 0.1	1.7 ± 0.6
18:0	21.2 ± 2.	17.5 ± 0.9	19.0 ± 1.3	23.5 ± 7.1	17.7 ± 1.3
18:1 (9)	15.5 ± 1.8^{a}	18.4 ± 0.8^{ab}	14.0 ± 1.4^{ab}	14.7 ± 1.0^{ab}	10.7 ± 0.6^{b}
18:1 (7)	1.5 ± 0.3	1.2 ± 0.3	1.1 ± 0.4	1.5 ± 0.4	1.3 ± 0.3
18:2(6)	4.5 ± 0.6^{a}	5.2 ± 0.7^{a}	3.2 ± 0.6^{b}	4.4 ± 0.8^{b}	3.4 ± 0.3^{b}
18:3 (6)	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1
19:0	0.2 ± 0.1	0.6 ± 0.5	2.8 ± 1.8	0.8 ± 0.01	0.7 ± 0.2
18:3 (3)	0.4 ± 0.2^{a}	0.4 ± 0.2^{ab}	0.7 ± 0.2^{ab}	0.6 ± 0.4^{ab}	1.0 ± 0.2^{b}
18:4 (3)	0.9 ± 0.3	0.3 ± 0.1	0.6 ± 0.4	0.8 ± 0.8	0.22 ±
20:0	0.5 ± 0.2	0.8 ± 0.4	1.7 ± 1.2	0.6 ± 0.2	0.4 ± 0.2
20:1 (9)	0.3 ± 0.1	0.2 ± 0.1	0.4 ± 0.2	0.6 ± 0.1	0.6 ± 0.1
20:1 (7)	1.4 ± 0.6	0.6 ± 0.1	1.0 ± 0.6	0.7 ± 0.1	0.9 ± 0.4
20:2 (6)	0.5 ± 0.2^{a}	1.8 ± 0.8^{b}	0.4 ± 0.2^{a}	0.7 ± 0.1^{ab}	0.7 ± 0.1^{ab}
20:3 (9)	0.5 ± 0.1	0.5 ± 0.2	0.3 ± 0.1	0.4 ± 0.2	0.6 ± 0.3
20:3 (6)	2.7 ± 1.9	1.7 ± 0.5	1.1 ± 0.3	1.7 ± 0.5	2.3 ± 1.0
20:4 (6)	9.2 ± 4.7^{a}	24.2 ± 4.3^{b}	22.7 ± 4.7^{ab}	20.8 ± 7.7^{ab}	30.9 ± 5.8^{b}
20:5 (3)	0.3 ± 0.2^{a}	0.3 ± 0.3^{a}	0.5 ± 0.3^{ab}	0.4 ± 0.4^{ab}	1.4 ± 0.3^{b}
22:0	0.2 ± 0.2	0.3 ± 0.1	0.4 ± 0.2	0.2 ± 0.1	1.0 ± 0.7
22:1 (9)	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.0	0.5 ± 0.3
22:3 (6)	nil*	nil*	nil*	0.1 ± 0.1	0.1 ± 0.1
22:4 (6)	1.8 ± 0.6	3.1 ± 1.0	3.2 ± 1.0	4.9 ± 2.2	4.6 ± 1.1
22:5 (6)	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.5 ± 0.1	0.3 ± 0.1
22:5 (3)	1.6 ± 0.5	2.0 ± 0.4	1.8 ± 0.4	1.9 ± 0.5	1.9 ± 0.5
24:0	0.3 ± 0.3	0.2 ± 0.1	0.5 ± 0.3	0.7 ± 0.3	0.3 ± 0.1
22:6 (3)	1.7 ± 0.5	1.7 ± 0.4	2.3 ± 0.8	2.0 ± 0.3	1.8 ± 0.6
24:1 (9)	0.1 ± 0.1	1.7 ± 1.6	0.3 ± 0.3	0.6 ± 0.5	0.7 ± 0.5
Σn-6	23 ± 4^{a}	33 ± 3^{ab}	33 ± 4^{ab}	30 ± 7^{ab}	38 ± 5^{b}
Σn-3	8 ± 1	5 ± 1	6 ± 1	7 ± 4	6 ± 2
ΣPUFA	34 ± 3^{a}	14 ± 3^{ab}	45 ± 3^{b}	37 ± 7^{ab}	44 ± 4^{ab}
ΣSFA	45 ± 3^{a}	34 ± 3^{b}	37 ± 3^{b}	45 ± 6^{ab}	41 ± 4^{ab}
ΣMUFA	19 ± 1^{ab}	22 ± 1^{a}	19 ± 1^{ab}	18 ± 3^{ab}	16 ± 2^{b}

*nil=<0.04%

TABLE IV.AP4: Fatty acid composition of the PE fraction of lymphocytes at post burn timepoints expressed a percent of total fatty acids. Data are expressed as means ± SEM (n ≤10). Significant differences between post burn timepoints were determined using a repeated measures ANOVA and values within a row not sharing common superscripts are significantly ($p<0.05$) different.

Appendix IV. AP5

Fatty Acid	*PS*	*PE*	*PI*	*PC*
	% fatty acids			
14:0	2.4 ± 0.7	1.9 ± 0.5	2.4 ± 1.0	0.8 ± 0.03
14:1(9)	nil*	nil*	0.3 ± 0.2	nil*
15:0	0.7 ± 0.2	2.2 ± 1.0	1.4 ± 1.0	0.5 ± 0.5
16:0	27.4 ± 10.0	21.5 ± 5.3	19.8 ± 7.5	30.5 ± 1.8
16:1(7)	1.5 ± 0.5	0.7 ± 0.2	1.1 ± 0.9	0.1 ± 0.1
16:1(5)	0.4 ± 0.2	0.7 ± 0.2	0.6 ± 0.3	0.4 ± 0.2
17:0	nil*	nil*	0.6 ± 0.4	nil*
18:0	28.0 ± 5.5	20.6 ± 2.4	24.4 ± 6.4	12.7 ± 0.9
18:1(9)	19.1 ± 3.0	14.9 ± 2.1	6.5 ± 1.3	13.5 ± 6.2
18:1(7)	0.8 ± 0.6	1.2 ± 0.2	1.9 ± 0.95	3.35 ± 1.70
18:2(6)	3.5 ± 0.8	3.6 ± 1.0	2.74 ± 0.64	7.13 ± 0.32
18:3(6)	nil*	nil*	0.60 ± 0.60	nil*
19:0	0.39 ± 0.26	0.81 ± 0.45	0.66 ± 0.48	1.40 ± 1.40
18:3(3)	0.99 ± 0.78	0.52 ± 0.36	1.05 ± 0.62	0.19 ± 0.19
18:4(3)	nil*	0.14 ± 0.11	0.29 ± 0.29	0.15 ± 0.15
20:0	nil*	0.40 ± 0.20	0.12 ± 0.12	0.85 ± 0.12
20:1(9)	0.73 ± 0.38	0.38 ± 0.28	nil*	nil*
20:1(7)	0.12 ± 0.12	0.32 ± 0.24	0.11 ± 0.11	nil*
20:2(6)	2.44 ± 1.12	0.49 ± 0.25	0.61 ± 0.44	0.19 ± 0.19
20:3(9)	0.12 ± 0.12	0.26 ± 0.15	0.18 ± 0.11	nil*
20:3(6)	0.45 ± 0.45	1.33 ± 0.45	0.93 ± 0.62	1.74 ± 0.10
20:4(6)	6.59 ± 3.32	22.12 ± 4.6	27.92 ± 2.3	10.17 ± 0.94
20:5(3)	0.23 ± 0.23	0.29 ± 0.14	1.62 ± 1.01	5.64 ± 3.31
22:0	0.18 ± 0.18	0.21 ± 0.18	0.35 ± 0.20	0.00 ± 0.00
22:1(9)	nil*	nil*	nil*	nil*
22:1(7)	nil*	nil*	0.20 ± 0.20	nil*
22:4(6)	0.73 ± 0.42	2.98 ± 1.09	0.27 ± 0.16	1.26 ± 0.61
22:5(6)	nil*	0.20 ± 0.10	1.18 ± 0.69	5.08 ± 2.62
22:5(3)	0.16 ± 0.16	1.86 ± 0.80	0.48 ± 0.31	0.63 ± 0.23
24:0	nil*	nil*	nil*	nil*
22:6(3)	0.81 ± 0.14	1.55 ± 0.67	3.29 ± 2.37	1.64 ± 1.27
24:1(9)	nil*	nil*	nil*	nil*
Σn-6	15.5 ± 1.3	32.3 ± 1.8	34.3 ± 2.3	25.6 ± 1.9
Σn-3	2.1 ± 0.9	4.3 ± 1.3	5.5 ± 2.1	2.6 ± 1.8
ΣPUFA	18.4 ± 1.2	37.6 ± 6.0	12.7 ± 2.5	33.8 ± 7.2
ΣSFA	60.9 ± 5.7	48.9 ± 7.1	50.8 ± 6.5	48.8 ± 0.8
ΣMUFA	22.8 ± 2.7	18.2 ± 2.4	10.5 ± 1.7	22.6 ± 2.6

*nil=<0.04%

TABLE IV.AP5: Fatty acid composition major PL fractions of lymphocytes isolated from healthy subjects expressed a percent of total fatty acids. Data are expressed as means ± SEM (n ≤ 6).

Appendix V.AP1

Fatty acid	t1	t2	t3	t4	t5
	(μg/ml plasma)				
C 14:0	4.3 ± 1.8	3.7 ± 0.9	4.5 ± 1.2	6.3 ± 2.6	3.8 ± 0.6
C 14:1(9)	1.4 ± 0.4^{a}	1.6 ± 0.6^{ab}	2.0 ± 0.4^{ab}	2.4 ± 1.7^{ab}	3.3 ± 0.7^{b}
C 15:0	1.4 ± 0.2	2.0 ± 0.4	2.1 ± 0.2	1.7 ± 0.6	2.0 ± 0.3
C 15:1	0.9 ± 0.2^{ab}	0.8 ± 0.2^{a}	1.6 ± 0.5^{ab}	1.0 ± 0.2^{ab}	1.7 ± 0.4^{b}
C 16:0	134.5 ± 6.5	147.2 ± 14.8	161.8 ± 21.5	147.5 ± 39.1	159.8 ± 13.6
C 16:1(7)	0.9 ± 0.2^{a}	1.0 ± 0.3^{ab}	1.4 ± 0.1^{ab}	0.8 ± 0.4^{ab}	1.8 ± 0.6^{b}
C 16:1(5)	3.2 ± 0.4	3.5 ± 0.7	3.9 ± 0.8	2.5 ± 0.2	3.1 ± 0.3
C 18:0	72.9 ± 3.3	80.7 ± 8.7	81.9 ± 10.7	80.1 ± 17.4	90.7 ± 11.6
C 18:1(9)	61.6 ± 3.3	61.2 ± 7.0	59.0 ± 10.6	57.8 ± 13.7	50.1 ± 7.9
C 18:1(7)	10.3 ± 0.8	9.6 ± 1.1	10.1 ± 1.3	8.1 ± 1.0	12.3 ± 1.8
C 18:2(6)	79.2 ± 4.0	82.8 ± 10.5	85.2 ± 12.2	84.9 ± 18.5	98.2 ± 14.3
C 18:3(6)	0.3 ± 0.1	0.7 ± 0.2	0.5 ± 0.2	0.4 ± 0.2	0.6 ± 0.3
C 19:0	1.3 ± 0.1^{a}	1.5 ± 0.4^{a}	1.4 ± 0.2^{a}	0.9 ± 0.3^{a}	3.9 ± 2.5^{b}
C 18:3 (3)	0.7 ± 0.2^{a}	0.5 ± 0.2^{a}	0.5 ± 0.2^{a}	0.9 ± 0.4^{ab}	2.0 ± 0.8^{b}
C 18:4(3)	0.9 ± 0.2	0.9 ± 0.3	0.9 ± 0.3	0.7 ± 0.3	0.7 ± 0.2
C 20:0	0.5 ± 0.1^{a}	0.8 ± 0.2^{a}	1.0 ± 0.2^{a}	0.7 ± 0.4^{a}	2.8 ± 1.4^{b}
C 20:1 (9)	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.2	0.2 ± 0.2	0.5 ± 0.2
C 20:1(7)	0.6 ± 0.1	0.6 ± 0.2	0.5 ± 0.1	0.6 ± 0.3	0.9 ± 0.2
C 20:2(6)	1.2 ± 0.3^{a}	2.5 ± 0.4^{b}	1.2 ± 0.3^{a}	2.0 ± 0.6^{ab}	2.2 ± 0.2^{ab}
C 20:3(9)	3.1 ± 1.2	2.5 ± 2.1	0.4 ± 0.2	0.6 ± 0.1	2.1 ± 1.4
C 20:3(6)	14.6 ± 3.0	21.0 ± 4.2	25.0 ± 3.9	20.9 ± 6.7	15.7 ± 3.4
C 20:4(6)	31.0 ± 3.8^{a}	35.8 ± 4.5^{ab}	46.8 ± 5.9^{b}	38.4 ± 6.7^{ab}	46.1 ± 6.0^{b}
C 20:5(3)	2.2 ± 0.3	2.3 ± 0.6	1.9 ± 0.6	1.6 ± 0.2	2.0 ± 0.8
C 22:1(9)	0.7 ± 0.3^{a}	0.8 ± 0.2^{ab}	1.3 ± 0.4^{a}	1.3 ± 0.7^{ab}	1.7 ± 0.5^{b}
C 22:1 (7)	0.4 ± 0.2	0.6 ± 0.2	0.7 ± 0.3	1.0 ± 0.3	0.9 ± 0.2
C 22:4(6)	0.9 ± 0.2^{a}	1.1 ± 0.1^{ab}	1.5 ± 0.3^{ab}	1.4 ± 0.5^{ab}	1.3 ± 0.2^{b}
C 22:5(6)	0.8 ± 0.1	0.8 ± 0.1	1.1 ± 0.3	1.0 ± 0.4	1.1 ± 0.4
C 22:5(3)	2.9 ± 0.5	2.7 ± 0.4	3.1 ± 0.6	2.5 ± 0.4	3.3 ± 0.6
C 22:6(3)	10.0 ± 0.9^{ab}	10.2 ± 1.2^{a}	11.5 ± 2.3^{ab}	14.4 ± 4.1^{b}	12.8 ± 2.5^{ac}
C 24:1(9)	1.7 ± 0.4	2.2 ± 0.5	1.9 ± 0.7	2.6 ± 0.9	2.0 ± 0.7
Σn-6	15 ± 2^{a}	18 ± 2^{ab}	22 ± 3^{abc}	24 ± 3^{bc}	25 ± 3^{c}
Σn-3	122 ± 14	144 ± 17	169 ± 22	147 ± 25	165 ± 22
ΣPUFA	142± 15	164 ± 18	190 ± 24	172 ± 27	191 ± 24
ΣSFA	79 ± 8	81 ± 9	86 ± 12	81 ± 14	81 ± 12
ΣMUFA	212 ± 19^{a}	225 ± 23^{ab}	295 ± 30^{b}	262 ± 34^{ab}	281 ± 30^{ab}

TABLE V.AP1: Fatty acid composition of plasma total phospholipids expressed as μg/ml plasma calculated using 17:0 (10 μg) as the standard. Data are expressed as means ± SEM (n ≤10). Significant differences between recovery timepoints were determined using a repeated measures ANOVA and values within a row not sharing common superscripts are significantly different ($p<0.05$).

Appendix V.AP2

Fatty acid	t1	t2	t3	t4	t5
	(µg/ml plasma)				
C 14:0	2.2 ± 0.4^{a}	4.9 ± 0.9^{b}	2.8 ± 1.0^{ab}	1.5 ± 1.0^{ab}	3.1 ± 1.0^{ab}
C 14:1(9)	2.7 ± 1.2	2.4 ± 0.6	4.2 ± 1.0	3.1 ± 1.5	3.6 ± 1.1
C 15:1	0.5 ± 0.1	0.3 ± 0.1	0.6 ± 0.1	0.6 ± 0.2	0.7 ± 0.2
C 16:0	54.8 ± 6.4^{a}	64.0 ± 5.0^{ab}	76.0 ± 5.6^{b}	66.7 ± 11.0^{ab}	76.8 ± 12.3^{b}
C 16:1(7)	2.1 ± 0.4	2.9 ± 0.5	1.9 ± 0.4	2.4 ± 0.9	3.6 ± 0.8
C 16:1(5)	9.6 ± 1.9	12.8 ± 1.9	16.9 ± 5.4	6.9 ± 1.6	12.0 ± 2.2
C 17:0	3.0 ± 0.4	2.7 ± 0.5	4.0 ± 1.0	4.1 ± 0.7	2.5 ± 0.2
C 18:0	14.7 ± 5.3	11.6 ± 1.2	13.0 ± 2.7	13.0 ± 2.5	15.0 ± 2.9
C 18:1(9)	73.1 ± 9.5	81.4 ± 7.9	83.2 ± 11.9	69.8 ± 19.7	88.2 ± 12.5
C 18:1(7)	4.6 ± 0.8^{a}	5.8 ± 0.7^{ab}	6.0 ± 0.5^{ab}	4.6 ± 1.3^{ab}	7.7 ± 1.6^{b}
C 18:2(6)	138.2 ± 20.3^{a}	162.5 ± 21.7^{ab}	170.3 ± 17.2^{ab}	177.2 ± 50.2^{ab}	215.8 ± 33.3^{b}
C 18:3(6)	2.4 ± 0.6	5.4 ± 1.1	4.3 ± 0.8	3.8 ± 1.7	5.9 ± 2.4
C 19:0	3.1 ± 0.9	2.8 ± 0.6	2.3 ± 0.4	2.3 ± 1.5	1.3 ± 0.4
C 18:3(3)	0.9 ± 0.5^{a}	0.6 ± 0.3^{a}	1.7 ± 0.7^{ab}	1.0 ± 0.6^{ab}	2.6 ± 0.8^{b}
C 18:4(3)	0.9 ± 0.1	1.0 ± 0.2	0.8 ± 0.1	0.6 ± 0.2	1.0 ± 0.1
C 20:0	0.6 ± 0.2^{a}	0.6 ± 0.2^{a}	1.5 ± 0.3^{b}	0.9 ± 0.4^{ab}	1.0 ± 0.3^{ab}
C 20:1(9)	0.2 ± 0.1^{ab}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.7 ± 0.7^{b}	0.1 ± 0.1^{ab}
C 20:1(7)	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.2	0.2 ± 0.1	0.2 ± 0.1
C 20:2(6)	0.2 ± 0.1^{ab}	0.3 ± 0.1^{ab}	0.3 ± 0.0^{a}	0.0 ± 0.0^{b}	0.2 ± 0.1^{ab}
C 20:3(9)	1.2 ± 0.3	1.3 ± 0.7	2.3 ± 0.8	1.2 ± 1.1	0.5 ± 0.3
C 20:3(6)	1.7 ± 0.7	2.5 ± 0.8	2.4 ± 0.7	3.3 ± 1.6	3.6 ± 1.0
C 20:4(6)	16.3 ± 3.0^{a}	17.7 ± 3.2^{a}	23.5 ± 4.2^{ab}	24.2 ± 5.3^{ab}	33.5 ± 4.7^{b}
C 20:5(3)	1.7 ± 0.4	2.1 ± 0.6	1.2 ± 0.3	0.9 ± 0.3	2.0 ± 0.4
C 22:0	0.4 ± 0.1	0.5 ± 0.2	0.8 ± 0.2	0.3 ± 0.2	0.6 ± 0.3
C 22:1(9)	0.5 ± 0.1	0.7 ± 0.2	0.4 ± 0.1	0.3 ± 0.2	0.5 ± 0.1
C 22:1(7)	0.3 ± 0.1	0.4 ± 0.1	0.6 ± 0.2	0.4 ± 0.2	0.9 ± 0.2
C 22:4(6)	0.3 ± 0.1^{a}	0.4 ± 0.2^{a}	0.7 ± 0.2^{ab}	0.2 ± 0.1^{a}	0.6 ± 0.4^{b}
C 22:5(6)	0.4 ± 0.1	0.6 ± 0.4	0.7 ± 0.4	0.8 ± 0.5	0.4 ± 0.3
C 22:5(3)	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.2	0.2 ± 0.2
C 22:6(3)	1.1 ± 0.3	1.7 ± 0.4	1.6 ± 0.2	0.9 ± 0.3	2.0 ± 0.3
C 24:1(9)	0.6 ± 0.2	0.6 ± 0.3	0.4 ± 0.2	0.7 ± 0.4	0.2 ± 0.1
Σn-6	5 ± 1^{a}	5 ± 1^{a}	6 ± 1^{a}	5 ± 1^{a}	9 ± 1^{b}
Σn-3	171 ± 20^{a}	190 ± 21^{a}	217 ± 27^{ab}	195 ± 34^{ab}	271 ± 30^{b}
ΣPUFA	165 ± 20^{a}	197 ± 19^{ab}	225 ± 25^{ab}	211 ± 31^{ab}	259 ± 28^{b}
ΣSFA	101 ± 9	104 ± 9	113 ± 12	109 ± 15	131 ± 13
ΣMUFA	71 ± 7^{a}	92 ± 7^{b}	105 ± 9^{b}	95 ± 12^{ab}	105 ± 10^{b}

TABLE V.AP2: Fatty acid composition of plasma CE expressed as µg/ml plasma calculated using 17:0 (10 µg) as the standard. Data are expressed as means ± SEM (n ≤10). Significant differences between recovery timepoints were determined using a repeated measures ANOVA and values within a row not sharing common superscripts are significantly different ($p<0.05$).

Appendix V.AP3

Fatty acid	t1	t2	t3	t4	t5
			(µg/ml plasma)		
C 14:0	6.0 ± 3.1	6.5 ± 2.1	9.1 ± 3.6	3.5 ± 1.8	6.2 ± 2.4
C 14:1(9)	4.5 ± 1.4	5.0 ± 1.0	3.1 ± 1.1	4.5 ± 2.5	4.4 ± 1.7
C 15:1	4.0 ± 2.1	2.6 ± 2.1	0.4 ± 0.1	0.3 ± 0.1	0.6 ± 0.0
C 16:0	153.4 ± 23.5	154.9 ± 23.9	148.1 ± 32.6	124.1 ± 23.1	129.0 ± 24.2
C 16:1(7)	8.8 ± 2.6	5.7 ± 2.0	4.0 ± 0.7	4.1 ± 1.5	3.9 ± 0.8
C 16:1(5)	15.2 ± 2.5	19.1 ± 3.9	20.8 ± 5.9	7.1 ± 1.4	15.9 ± 3.8
C 17:0	3.3 ± 0.7	7.6 ± 4.2	3.4 ± 0.4	3.8 ± 0.2	2.9 ± 0.4
C 18:0	34.3 ± 6.5	30.1 ± 4.9	25.3 ± 4.4	23.1 ± 3.6	23.5 ± 2.9
C 18:1(9)	224.5 ± 31.9	213.7 ± 35.1	193.4 ± 39.2	144.2 ± 24.5	162.7 ± 33.3
C 18:1(7)	15.4 ± 2.3	15.1 ± 3.3	16.3 ± 3.9	8.5 ± 0.6	13.6 ± 3.3
C 18:2(6)	98.7 ± 23.4	90.1 ± 19.5	87.5 ± 23.4	93.7 ± 26.0	88.8 ± 24.0
C 18:3(6)	1.7 ± 0.4	3.1 ± 0.7	1.4 ± 0.6	2.5 ± 1.0	3.7 ± 1.8
C 19:0	2.1 ± 0.6	2.6 ± 0.6	3.2 ± 1.0	2.4 ± 0.8	2.3 ± 0.8
C 18:3 (3)	22.1 ± 11.3	12.1 ± 11.0	1.6 ± 0.5	2.7 ± 1.1	3.2 ± 1.6
C 18:4(3)	5.0 ± 2.1	2.5 ± 0.9	2.0 ± 0.4	0.9 ± 0.3	1.0 ± 0.3
C 20:0	1.5 ± 0.5	1.0 ± 0.3	0.6 ± 0.1	1.3 ± 0.8	0.8 ± 0.1
C 20:1 (9)	4.0 ± 1.8	1.6 ± 0.8	0.9 ± 0.4	0.4 ± 0.1	0.5 ± 0.1
C 20:1(7)	1.0 ± 0.3	0.9 ± 0.2	0.5 ± 0.1	2.1 ± 1.4	0.9 ± 0.3
C 20:2(6)	0.7 ± 0.2	0.9 ± 0.3	1.4 ± 0.6	1.5 ± 0.4	1.0 ± 0.4
C 20:3(9)	0.6 ± 0.3	1.4 ± 0.8	0.9 ± 0.6	0.0 ± 0.0	0.8 ± 0.3
C 20:3(6)	3.0 ± 1.3	1.2 ± 0.4	1.2 ± 0.6	2.1 ± 0.7	1.1 ± 0.5
C 20:4(6)	11.0 ± 3.5	8.0 ± 2.2	7.2 ± 2.6	6.3 ± 1.4	6.7 ± 2.1
C 20:5(3)	0.7 ± 0.3	0.6 ± 0.2	0.6 ± 0.3	0.4 ± 0.1	0.6 ± 0.2
C 22:0	2.9 ± 1.5	1.3 ± 1.0	0.2 ± 0.1	0.9 ± 0.7	0.2 ± 0.1
C 22:1(9)	1.1 ± 0.4	0.9 ± 0.4	0.4 ± 0.1	0.3 ± 0.1	0.7 ± 0.1
C 22:1 (7)	0.9 ± 0.4	0.8 ± 0.4	0.4 ± 0.1	0.7 ± 0.1	0.5 ± 0.1
C 22:4(6)	0.8 ± 0.2	0.7 ± 0.1	0.8 ± 0.3	0.8 ± 0.3	0.8 ± 0.2
C 22:5(6)	4.7 ± 1.6	2.1 ± 0.8	1.4 ± 0.2	0.7 ± 0.3	2.0 ± 0.8
C 22:5(3)	1.8 ± 0.5	0.9 ± 0.2	0.9 ± 0.2	0.5 ± 0.2	1.0 ± 0.3
C 22:6(3)	1.5 ± 0.3	1.4 ± 0.3	1.8 ± 0.5	0.8 ± 0.3	1.7 ± 0.3
C 24:1(9)	0.1 ± 0.1	0.6 ± 0.4	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.1
Σn-6	6.5 ± 1.3	5.0 ± 1.4	7.0 ± 1.7	5.8 ± 2.2	8.1 ± 1.9
Σn-3	88.5 ± 13.2	78.2 ± 14.3	102.8 ± 17.2	100.9 ± 21.7	78.7 ± 19.3
ΣPUFA	80.3 ± 13.0	96.0 ± 14.1	113.1 ± 17.0	103.3 ± 21.4	122.4 ± 19.0
ΣSFA	247.5 ± 27.7	258.9 ± 30.1	247.9 ± 36.2	197.0 ± 45.7	231.1 ± 40.6
ΣMUFA	164.1 ± 21.6	189.9 ± 23.5	162.1 ± 28.2	162.7 ± 35.6	188.3 ± 31.6

TABLE V.AP3: Fatty acid composition of plasma TG expressed as µg/ml plasma calculated using 15:0 (10 µg) as the standard. Data are expressed as means ± SEM (n ≤10). Significant differences between recovery timepoints were determined using a repeated measures ANOVA and values within a row not sharing common superscripts are significantly different ($p<0.05$).

Appendix V.AP4

Fatty Acid	*PL (% FA)*	*PL (μg/ml)*	*CE (%)*	*CE (μg/ml)*	*TG (%)*	*TG (μg/ml)*
C 14:0	1.0 ± 0.4	5.9 ± 2.9	0.4 ± 0.1	2.1 ± 0.6	0.7 ± 0.2	4.7 ± 1.5
C 14:1(9)	0.2 ± 0.0	1.7 ± 0.5	0.7 ± 0.2	6.7 ± 2.7	1.1 ± 0.5	6.0 ± 2.2
C 16:0	28.4 ± 1.1	206.5 ± 31.0	13.1 ± 2.0	107.4 ± 23.4	23.1 ± 2.0	148.1 ± 22.5
C 16:1(7)	0.2 ± 0.0	1.5 ± 0.2	0.4 ± 0.1	2.4 ± 0.4	1.4 ± 0.5	8.9 ± 3.2
C 16:1(5)	0.4 ± 0.1	3.0 ± 0.6	2.0 ± 0.4	15.6 ± 3.5	2.4 ± 0.4	15.3 ± 3.0
C 18:0	14.4 ± 1.0	98.8 ± 10.9	4.0 ± 1.7	36.9 ± 21.3	4.6 ± 0.6	31.8 ± 5.0
C 18:1(9)	10.0 ± 0.8	70.2 ± 9.8	19.0 ± 1.8	134.7 ± 17.0	36.0 ± 2.4	262.5 ± 59.1
C 18:1(7)	2.1 ± 0.2	14.7 ± 2.5	1.2 ± 0.1	9.2 ± 1.4	3.0 ± 0.5	21.2 ± 5.3
C 18:2(6)	23.1 ± 1.7	174.7 ± 29.8	49.8 ± 2.3	372.9 ± 51.7	16.9 ± 2.6	120.4 ± 30.4
C 18:3(6)	0.1 ± 0.0	0.6 ± 0.1	0.5 ± 0.2	3.4 ± 0.8	0.3 ± 0.1	2.4 ± 0.6
C 19:0	0.6 ± 0.4	2.7 ± 1.3	0.5 ± 0.1	2.9 ± 0.8	0.7 ± 0.1	4.1 ± 1.0
C 18:3(3)	0.2 ± 0.1	1.5 ± 0.6	0.2 ± 0.1	1.5 ± 0.9	2.6 ± 2.1	19.6 ± 13.1
C 18:4(3)	0.2 ± 0.0	0.8 ± 0.2	0.2 ± 0.0	1.1 ± 0.2	0.5 ± 0.2	3.3 ± 1.1
C 20:0	0.5 ± 0.3	2.1 ± 1.1	0.0 ± 0.0	0.3 ± 0.1	0.2 ± 0.1	1.7 ± 0.6
C 20:1 (9)	0.1 ± 0.0	0.5 ± 0.2	0.0 ± 0.0	0.1 ± 0.1	0.3 ± 0.2	2.2 ± 1.0
C 20:1(7)	0.1 ± 0.0	0.8 ± 0.2	0.1 ± 0.0	0.8 ± 0.4	0.2 ± 0.1	1.6 ± 0.9
C 20:2(6)	0.3 ± 0.1	2.0 ± 0.5	0.10.0 ±	0.5 ± 0.2	0.2 ± 0.0	0.9 ± 0.4
C 20:3(9)	0.4 ± 0.3	2.8 ± 1.8	0.3 ± 0.1	2.3 ± 0.6	0.2 ± 0.0	0.9 ± 0.4
C 20:3(6)	2.5 ± 0.6	16.8 ± 3.2	0.2 ± 0.1	1.7 ± 1.0	0.2 ± 0.1	1.7 ± 0.6
C 20:4(6)	9.1 ± 0.4	67.9 ± 11.2	5.3 ± 0.3	39.3 ± 5.4	1.1 ± 0.3	8.5 ± 1.9
C 20:5(3)	0.3 ± 0.1	1.5 ± 0.4	0.4 ± 0.1	2.8 ± 0.6	0.1 ± 0.0	0.7 ± 0.3
C 22:0	0.4 ± 0.2	2.1 ± 0.7	0.0 ± 0.0	0.3 ± 0.1	0.3 ± 0.2	1.5 ± 1.2
C 22:1(9)	0.2 ± 0.1	1.8 ± 0.9	0.1 ± 0.0	0.5 ± 0.1	0.2 ± 0.0	1.3 ± 0.5
C 22:1 (7)	0.0 ± 0.0	0.2 ± 0.1	0.0 ± 0.0	0.2 ± 0.1	0.0 ± 0.0	0.4 ± 0.3
C 22:4(6)	0.3 ± 0.0	2.2 ± 0.3	0.0 ± 0.0	0.3 ± 0.1	0.3 ± 0.1	1.2 ± 0.2
C 22:5(6)	0.2 ± 0.0	1.7 ± 0.4	0.1 ± 0.0	0.6 ± 0.3	0.8 ± 0.4	6.6 ± 3.4
C 22:5(3)	0.6 ± 0.1	4.1 ± 0.6	0.0 ± 0.0	0.2 ± 0.2	0.2 ± 0.0	1.5 ± 0.4
C 22:6(3)	2.8 ± 0.2	20.6 ± 3.2	0.4 ± 0.1	3.1 ± 0.6	0.4 ± 0.0	3.0 ± 0.6
C 24:1(9)	0.3 ± 0.1	2.8 ± 1.0	0.1 ± 0.0	0.6 ± 0.3	0.0 ± 0.0	0.1 ± 0.1

TABLE V.AP4: Fatty acid composition of plasma PL, CE and TG isolated from healthy subjects (n ≤ 6) expressed as percent of total fatty acids and as μg/ml plasma calculated using 17:0 (10 μg; PL and CE) 15:0 (10 μg; TG) as the standards. Data are expressed as means ± SEM (n ≤8).

Appendix V.AP5

Fatty acid	*t1*	*t2*	*t3*	*t4*	*t5*
			(% fatty acids)		
C 14:0	0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
C 15:0	0.1 ± 0.02	0.2 ± 0.1	0.1 ± 0.02	0.1 ± 0.1	0.2 ± 0.1
C 16:0	42.0 ± 0.7	40.4 ± 0.8	40.6 ± 1.2	43.1 ± 0.5	39.0 ± 1.2
C 16:1(7)	1.1 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	0.7 ± 0.2	0.8 ± 0.1
C 16:1(5)	0.1 ± 0.1	nil*	nil*	0.2 ± 0.2	nil*
C 17:0	0.2 ± 0.02	0.2 ± 0.02	0.1 ± 0.03	0.1 ± 0.03	0.2 ± 0.02
C 18:0	10.2 ± 0.1	10.2 ± 0.3	10.7 ± 0.6	11.3 ± 0.2	10.7 ± 0.7
C 18:1(9)	20.3 ± 0.4	18.7 ± 0.5	19.6 ± 0.9	20.2 ± 0.6	19.8 ± 0.6
C 18:1(7)	2.96 ± 0.05	2.89 ± 0.18	2.68 ± 0.15	2.73 ± 0.15	2.93 ± 0.04
C 18:2(6)	16.9 ± 0.4	18.5 ± 0.6	15.6 ± 1.1	15.3 ± 0.6	18.0 ± 1.3
C 18:3(6)	0.1 ± 0.01	0.1 ± 0.02	0.1 ± 0.01	0.1 ± 0.02	0.1 ± 0.03
C 19:0	0.1 ± 0.02	nil*	nil*	nil*	0.1 ± 0.1
C 18:3(3)	0.2 ± 0.03	0.2 ± 0.02	0.2 ± 0.03	0.1 ± 0.01	0.2 ± 0.04
C 18:4(3)	0.2 ± 0.01	0.2 ± 0.03	0.2 ± 0.04	0.2 ± 0.03	0.2 ± 0.01
C 20:0	0.1 ± 0.01	0.1 ± 0.00	0.1 ± 0.1	0.1 ± 0.01	0.1 ± 0.07
C 20:1(9)	0.2 ± 0.01	0.2 ± 0.02	0.3 ± 0.01	0.3 ± 0.01	0.2 ± 0.02
C 20:1(7)	0.1 ± 0.02	0.1 ± 0.03	0.1 ± 0.03	0.1 ± 0.04	0.1 ± 0.03
C 20:2(6)	0.3 ± 0.02	0. ± 0.02	0.3 ± 0.04	0.4 ± 0.04	0.4 ± 0.03
C 20:3(9)	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.00	0.1 ± 0.01
C 20:3(6)	1.2 ± 0.1	2.0 ± 0.2	2.0 ± 0.3	1.7 ± 0.3	1.6 ± 0.2
C 20:4(6)	2.2 ± 0.4^{a}	3.2 ± 0.3^{ab}	3.2 ± 0.3^{ab}	2.8 ± 0.5^{ab}	4.0 ± 0.4^{b}
C 20:5(3)	0.2 ± 0.02	0.2 ± 0.05	0.2 ± 0.07	0.1 ± 0.01	0.3 ± 0.10
C 22:4(6)	0.1 ± 0.02	0.1 ± 0.02	0.1 ± 0.02	0.1 ± 0.02	0.1 ± 0.1
C 22:5(3)	0.1 ± 0.02	0.2 ± 0.03	0.17 ± 0.04	0.07 ± 0.01	0.21 ± 0.06
C 22:6(3)	0.4 ± 0.04	0.6 ± 0.1	1.1 ± 0.5	0.3 ± 0.1	0.7 ± 0.2
C 24:1(9)	0.1 ± 0.1	0.1 ± 0.02	0.8 ± 0.7	0.2 ± 0.1	nil*
Σ N6	21.6 ± 1.2^{ab}	24.2 ± 1.0^{a}	23.0 ±1.1^{ab}	19.8 ± 1.7^{b}	23.9 ±1.5^{ab}
Σ N3	1.0 ± 0.1^{a}	1.2 ± 1.2^{ab}	1.3 ± 0.1^{ab}	1.1 ± 0.2^{ab}	1.6 ± 0.2^{b}
Σ SFA	53.2 ± 1.0^{ab}	51.9 ± 0.9	52.7 ± 0.9	54.2 ± 1.4	50.1 ± 1.2
Σ MUFA	24.7 ± 0.7^{a}	22.7 ± 0.5^{b}	24.4 ± 0.6^{ab}	24.5 ± 0.9^{ab}	24.0 ± 0.8^{b}

nil* = ≤0.04%

Table V.AP5: Fatty acid composition of the PC fraction of erythrocytes at post burn timepoints expressed a percent of total fatty acids. Data are expressed as means ± SEM (n ≤10). Significant differences between post burn timepoints were determined using a repeated measures ANOVA and values within a row not sharing common superscripts are significantly ($p<0.05$) different.

Appendix V.AP6

Fatty Acid	*t1*	*t2*	*t3*	*t4*	*t5*
			(% fatty acids)		
C 14:0	0.3 ± 0.03	0.4 ± 0.1	0.3 ± 0.1	0. 6 ± 0.1	0.2 ± 0.03
C 15:0	0.2 ± 0.02	0.1 ± 0.03	0.2 ± 0.1	0.2 ± 0.1	0.5 ± 0.3
C 16:0	21.8 ± 0.5	20.8 ± 0.9	20.4 ± 1.1	24.8 ± 1.5	20.1 ± 1.4
C 16:1(7)	0.7 ± 0.1	0.8 ± 0.2	0.6 ± 0.2	0.4 ± 0.2	0.5 ± 0.1
C 16:1(5)	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.6 ± 0.2	0.2 ± 0.2
C 17:0	5.5 ± 0.8	4.9 ± 1.3	5.0 ± 1.3	2.2 ± 0.8	4.6 ± 1.1
C 18:0	9.0 ± 0.4	8.9 ± 0.5	8.5 ± 0.8	10.4 ± 0.7	8.2 ± 1.1
C 18:1(9)	29.9 ± 1.0	26.4 ± 1.6	25.7 ± 1.6	30.8 ± 1.7	26.2 ± 2.3
C 18:1(7)	2.1 ± 0.3	2.4 ± 0.2	1.9 ± 0.2	2.7 ± 0.3	2.2 ± 0.4
C 18:2(6)	7.1 ± 0.1	7.4 ± 0.3	7.0 ± 0.5	8.0 ± 0.6	7.3 ± 0.5
C 19:0	0.1 ± 0.02	nil*	0.36 ± 0.27	0.1 ± 0.1	0.2 ± 0.1
C 18:3(3)	0.5 ± 0.04	0.46 ± 0.02	0.6 ± 0.1	0.4 ± 0.1	0.5 ± 0.1
C 18:4(3)	0.3 ± 0.1	0.20 ± 0.1	0.2 ± 0.04	0.1 ± 0.1	0.2 ± 0.04
C 20:0	0.1 ± 0.02	0.05 ± 0.01	0.4 ± 0.3	0.2 ± 0.1	0.1 ± 0.04
C 20:1(9)	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
C 20:1(7)	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.2	0.3 ± 0.2
C 20:2(6)	0.4 ± 0.01	0.4 ± 0.02	0.4 ± 0.04	0.5 ± 0.06	0.4 ± 0.1
C 20:3(9)	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.02	0.1 ± 0.01	0.1 ± 0.01
C 20:3(6)	1.0 ± 0.1	1.2 ± 0.1	1.4 ± 0.2	1.3 ± 0.2	1.4 ± 0.2
C 20:4(6)	13.2 ± 1.6^{a}	15.4 ± 1.3^{ab}	17.9 ± 1.3^{b}	11.9 ± 2.0^{a}	17.8 ± 1.8^{ab}
C 20:5(3)	0.5 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.3 ± 0.03	0.7 ± 0.3
C 22:1(9)	0.1 ± 0.02	0.1 ± 0.02	0.1 ± 0.02	0.1 ± 0.02	0.1 ± 0.01
C 22:4(6)	2.7 ± 0.4	3.0 ± 0.4	3.5 ± 0.6	1.8 ± 0.6	3.2 ± 1.0
C 22:5(6)	0.3 ± 0.03	0.4 ± 0.04	0.4 ± 0.06	0.2 ± 0.04	0.4 ± 0.1
C 22:5(3)	1.7 ± 0.2	2.0 ± 0.3	2.1 ± 0.3	1.2 ± 0.1	2.1 ± 0.4
C 22:6(3)	1.7 ± 0.2	2.5 ± 0.3	2.5 ± 0.3	1.4 ± 0.1	3.1 ± 0.8
C 22:1(9)	0.05 ± 0.02	0.05 ± 0.02	nil*	nil*	nil*
ΣN6	26.8 ± 1.9^{a}	28.8 ± 1.6^{ab}	32.7 ± 1.6^{b}	24.0 ± 2.5^{a}	29.7 ± 2.2^{ab}
ΣN3	4.5 ± 0.7^{a}	5.6 ± 0.6^{ab}	5.9 ± 0.6^{ab}	4.2 ± 0.9^{a}	6.8 ± 0.8^{b}
ΣSFA	36.1 ± 1.0	35.7 ± 1.0	35.1 ± 1.0	36.9 ± 1.7	34.6 ± 1.4
ΣMUFA	34.0 ± 1.4^{a}	31.1 ± 1.4^{ab}	29.2 ± 1.4^{b}	32.8 ± 2.3^{ab}	29.2 ± 2.0^{ab}
ΣPUFA	29.8 ± 2.3^{a}	33.1 ± 2.4^{ab}	37.1 ± 2.4^{b}	29.7 ± 3.8^{ab}	35.9 ± 3.3^{abg}

nil* = ≤0.04%

Table V.AP5: Fatty acid composition of the PE fraction of erythrocytes at post burn timepoints expressed a percent of total fatty acids. Data are expressed as means ± SEM (n ≤10). Significant differences between post burn timepoints were determined using a repeated measures ANOVA and values within a row not sharing common superscripts are significantly ($p<0.05$) different.

Appendix V.AP7

Fatty acid	*t1*	*t2*	*t3*	*t4*	*t5*
			(% fatty acids)		
C 14:0	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.7 ± 0.4	0.4 ± 0.1
C 15:0	0.2 ± 0.0	0.16 ± 0.03	0.24 ± 0.05	0.22 ± 0.04	0.19 ± 0.10
C 16:0	14.7 ± 1.3	9.0 ± 1.3	11.3 ± 1.1	11.6 ± 2.5	10.2 ±
C 16:1(7)	0.4 ± 0.1	0.3 ± 0.06	0.36 ± 0.02	0.47 ± 0.13	0.26 ± 0.02
C 16:1(5)	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.2	0.2 ± 0.1
C 17:0	1.0 ± 0.3	0.4 ± 0.2	0.6 ± 0.1	0.6 ± 0.1	0.3 ± 0.1
C 18:0	34.3 ± 1.8	38.3 ± 1.3	34.1 ± 3.1	33.7 ± 4.9	38.7 ± 1.7
C 18:1(9)	15.6 ± 1.1	11.8 ± 1.1	12.6 ± 2.3	17.8 ± 2.4	11.1 ± 2.5
C 18:1(7)	2.2 ± 0.1	1.9 ± 0.2	1.7 ± 0.2	2.0 ± 0.1	2.0 ± 0.2
C 18:2(6)	4.7 ± 0.4	3.7 ± 0.3	4.2 ± 0.2	4.3 ± 0.4	3.5 ± 0.3
C 19:0	0.2 ± 0.04	0.1 ± 0.03	0.3 ± 0.1	0.1 ± 0.03	0.2 ± 0.1
C 18:3(3)	0.5 ± 0.1	0.3 ± 0.1	0.5 ± 0.2	0.4 ± 0.2	0.1 ± 0.1
C 18:4(3)	0.1 ± 0.0	0.1± 0.03	0.1 ± 0.03	0.1 ± 0.04	0.1 ± 0.02
C 20:0	0.3 ± 0.02	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
C 20:1(9)	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.2	0. 6 ± 0.2	0.4 ± 0.04
C 20:1(7)	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.2	0.1 ± 0.02
C 20:2(6)	0.3 ± 0.02	0.2 ± 0.1	0.3 ± 0.04	0.3 ± 0.04	0.3 ± 0.1
C 20:3(6)	1.6 ± 0.2	2.0 ± 0.2	2.2 ± 0.3	2.3 ± 0.4	2.3 ± 0.3
C 20:4(6)	14.5 ± 1.5^{a}	18.6 ± 1.2^{b}	15.9 ± 1.2^{ab}	15.3 ± 1.9^{ab}	20.1 ± 1.6^{b}
C 20:5(3)	0.2 ± 0.1	0.3 ± 0.03	0.4 ± 0.1	0.2 ± 0.04	0.3 ± 0.1
C 22:0	0.2 ± 0.04	0.3 ± 0.2	0.2 ± 0.1	0.1 ± 0.04	0.1 ± 0.03
C 22:1(9)	0.5 ± 0.2	0.4 ± 0.2	0.7 ± 0.4	0.6 ± 0.4	0.1 ± 0.04
C 22:4(6)	2.1 ± 0.3	2.7 ± 0.3	3.5 ± 0.6	3.1 ± 0.2	2.4 ± 0.7
C 22:5(6)	0.5 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.8 ± 0.2
C 22:5(3)	2.0 ± 0.2	2.6 ± 0.2	2.7 ± 0.3	1.9 ± 0.1	2.5 ± 0.5
C 22:6(3)	2.8 ± 0.3	4.3 ± 0.5	4.2 ± 0.5	2.8 ± 0.1	5.4 ± 1.4
C 22:1(9)	0.2 ± 0.05	0.1 ± 0.04	0.3 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
ΣN-6	25.8 ± 1.7	28.6 ± 1.4	29.0 ± 1.6	25.6 ± 2.4	29.6 ± 2.1
ΣN-3	5.7 ± 0.8	7.7 ± 0.7	7.3 ± 0.8	6.2 ± 1.2	8.1 ± 1.1
ΣPUFA	32.0 ± 2.5^{a}	36.9 ± 2.2^{ab}	39.8 ± 2.3^{b}	32.4 ± 3.5^{ab}	36.1 ± 3.1^{ab}
ΣSFA	49.8 ± 2.0	47.5 ± 1.7	46.7 ± 1.9	45.3 ± 2.8	48.9 ± 2.5
ΣMUFA	18.9 ± 1.1^{a}	15.6 ± 0.9^{bc}	14.8 ± 1.0^{bc}	18.4 ± 1.6^{ab}	13.6 ± 1.4^{c}

nil*= ≤0.04%

Table V.AP7: Fatty acid composition of the PS fraction of erythrocytes at post burn timepoints expressed a percent of total fatty acids. Data are expressed as means ± SEM (n ≤10). Significant differences between post burn timepoints were determined using a repeated measures ANOVA and values within a row not sharing common superscripts are significantly ($p<0.05$) different.

Appendix V.AP8

Fatty acid	*t1*	*t2*	*t3*	*t4*	*t5*
			(% fatty acids)		
C 14:0	0.7 ± 0.2	0.9 ± 0.2	0.9 ± 0.3	0.9 ± 0.4	0.8 ± 0.4
C 15:0	0.4 ± 0.1	0.5 ± 0.2	0.4 ± 0.1	0.2 ± 0.1	0.4 ± 0.1
C 16:0	24.6 ± 1.5	23.5 ± 1.2	27.2 ± 1.7	23.3 ± 0.9	24.0 ± 2.4
C 16:1(7)	0.6 ± 0.1	0.7 ± 0.2	0.5 ± 0.1	0.6 ± 0.2	0.8 ± 0.3
C 16:1(5)	0.5 ± 0.1	0.5 ± 0.2	0.4 ± 0.2	0.7 ± 0.4	0.4 ± 0.2
C 17:0	4.3 ± 1.2	1.7 ± 0.8	2.3 ± 0.6	2.3 ± 1.2	0.8 ± 0.3
C 18:0	23.0 ± 1.7	21.3 ± 1.4	20.4 ± 1.8	20.1 ± 1.3	25.7 ± 0.7
C 18:1(9)	16.5 ± 0.9	15.5 ± 1.5	18.0 ± 1.8	22.4 ± 3.1	15.2 ± 1.7
C 18:1(7)	2.9 ± 0.4	2.5 ± 0.2	2.5 ± 0.3	3.3 ± 0.8	2.8 ± 0.3
C 18:2(6)	6.7 ± 1.3	6.8 ± 0.4	6.7 ± 0.5	6.8 ± 0.4	5.7 ± 0.9
C 19:0	1.1 ± 0.4	1.5 ± 0.8	0.1 ± 0.1	0.2 ± 0.1	0.5 ± 0.3
C 18:3(3)	1.0 ± 0.2	1.6 ± 0.7	0.8 ± 0.2	0.6 ± 0.2	0.4 ± 0.1
C18:4(3)	0.3 ± 0.1	1.0 ± 0.7	0.4 ± 0.1	0.2 ± 0.1	0.5 ± 0.1
C 20:0	0.8 ± 0.3	1.2 ± 0.6	0.2 ± 0.1	0.3 ± 0.1	0.4 ± 0.1
C 20:1(9)	0.7 ± 0.1	0.4 ± 0.2	0.6 ± 0.2	0.3 ± 0.2	0.2 ± 0.1
C 20:1(7)	0.3 ± 0.1	0.6 ± 0.3	0.3 ± 0.1	0.8 ± 0.2	0.2 ± 0.1
C 20:2(6)	0.3 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.2	0.3 ± 0.2
C 20:3(9)	0.10 ± 0.04	0.1 ± 0.1	0.2 ± 0.2	0.4 ± 0.2	0.2 ± 0.1
C 20:3(6)	1.1 ± 0.2	1.5 ± 0.2	1.6 ± 0.3	1.8 ± 0.6	1.8 ± 0.4
C 20:4(6)	6.8 ± 0.7^{a}	8.6 ± 0.6^{ab}	9.0 ± 0.6^{b}	7.0 ± 0.9^{ab}	11.6 ± 0.8^{c}
C 20:5(3)	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	nil*	0.2 ± 0.1
C 22:0	0.6 ± 0.2	0.5 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	0.5 ± 0.1
C 22:1(9)	0.9 ± 0.3	1.4 ± 0.7	1.0 ± 0.5	0.7 ± 0.6	0.6 ± 0.3
C 22:1(7)	0.6 ± 0.2	0.7 ± 0.3	0.2 ± 0.1	0.4 ± 0.2	0.4 ± 0.3
C 22:4(6)	1.0 ± 0.1	1.7 ± 0.5	1.5 ± 0.2	1.2 ± 0.4	1.5 ± 0.4
C 22:5(6)	0.08 ± 0.04	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1
C 22:5(3)	0.7 ± 0.2	0.8 ± 0.3	0.6 ± 0.2	0.4 ± 0.2	0.9 ± 0.3
C 24:0	0.11 ± 0.09	0.08 ± 0.07	0.2 ± 0.1	nil*	nil*
C 22:6(3)	1.5 ± 0.2	1.8 ± 0.3	2.1 ± 0.3	1.4 ± 0.4	2.5 ± 0.6
C 24:1(9)	1.6± 0.3	1.1 ± 0.3	1.6 ± 0.3	2.4 ± 0.9	1.8 ± 0.5
ΣN-6	16.7 ± 1.6^{a}	18.5 ± 1.4^{ab}	21.4 ± 1.5^{b}	18.7 ± 2.3^{ab}	21.7 ± 2.0^{ab}
ΣN-3	3.8 ± 0.7	5.4 ± 0.6	4.2 ± 0.6	3.2 ± 1.0	4.6 ± 0.9
ΣSFA	56.4 ± 1.7^{a}	52.8 ± 1.4^{ab}	53.8 ± 1.5^{a}	47.5 ± 2.3^{b}	51.0 ± 2.1^{ab}
ΣMUFA	24.7 ± 1.2^{a}	23.5 ± 1.0^{a}	23.6 ± 1.1^{a}	29.6 ± 1.7^{b}	22.7 ± 1.5^{a}
ΣPUFA	19.9 ± 1.4^{a}	23.5 ± 1.2^{ab}	22.6 ± 1.3^{a}	20.6 ± 2.0^{a}	27.3 ± 1.8^{b}

nil* = ≤0.04%

Table V.AP8: Fatty acid composition of the PI fraction of erythrocytes at post burn timepoints expressed a percent of total fatty acids. Data are expressed as means ± SEM ($n \leq 10$). Significant differences between post burn timepoints were determined using a repeated measures ANOVA and values within a row not sharing common superscripts are significantly ($p<0.05$) different.

Appendix VII.AP1

Fatty Acid	***PL***		***CE***		***TG***	
	Harvest	Discharge	Harvest	Discharge	Harvest	Discharge
	(μg/ml plasma)		*(μg/ml plasma)*		*(μg/ml plasma)*	
C14:0	0.1 ± 0.1	0.0 ± 0.0	1.3 ± 0.7	1.2 ± 0.7	4.5 ± 2.3	3.9 ± 0.9
C16:0	73.7 ± 14.6	52.9 ± 7.7	30.1 ± 6.8	31.9 ± 1.5	99.9 ± 35.4	122.7 ± 19.1
C16:1	1.1 ± 0.6	0.9 ± 0.5	6.4 ± 1.4	6.7 ± 1.6	15.1 ± 5.7	19.0 ± 3.5
C17:0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1	0.4 ± 0.4	1.4 ± 0.7	2.8 ± 1.0
C17:1	0.7 ± 0.5	0.6 ± 0.3	0.4 ± 0.2	0.2 ± 0.2	1.1 ± 0.6	1.4 ± 0.1
C18:0	52.1 ± 16.1	32.4 ± 6.2	3.5 ± 0.7	5.3 ± 2.1	23.5 ± 10.0	23.5 ± 5.3
C18:1	40.8 ± 8.7	28.5 ± 4.3	68.7 ± 17.8	59.5 ± 6.0	257.3 ± 97.5	287.2 ± 48.9
C18:2 (6)	73.0 ± 22.7	37.6 ± 7.4	150.7 ± 52.4	88.2 ± 19.1	93.4 ± 38.3	87.4 ± 15.3
C18:3 (6)	nil*	0.2 ± 0.2	2.3 ± 1.0	0.9 ± 0.9	1.0 ± 0.5	1.6 ± 0.5
C18:3 (3)	1.1 ± 0.2	0.1 ± 0.1	2.1 ± 0.6	0.4 ± 0.4	7.9 ± 4.3	5.5 ± 2.1
C20:0	0.4 ± 0.3 ±	0.1 ± 0.1	nil*	nil*	0.9 ± 0.7	0.4 ± 0.4
C20:1 (9)	0.9 ± 0.3	0.3 ± 0.2	nil*	nil*	3.3 ± 1.9	2.2 ± 0.6
C20:2 (6)	2.7 ± 0.7	0.7 ± 0.2	0.8 ± 0.2	0.2 ± 0.2	8.3 ± 0.7	7.0 ± 0.2
C20:3 (6)	14.1 ± 5.5	6.9 ± 2.5	2.7 ± 1.2	1.3 ± 0.7	1.5 ± 0.8	1.0 ± 0.5
C20:4 (6)	43.1 ± 13.8	34.6 ± 9.2	21.6 ± 8.4	23.2 ± 8.8	7.5 ± 2.0	12.4 ± 3.6
C20:5 (3)	1.3 ± 0.9	0.1 ± 0.1	0.9 ± 0.2	0.2 ± 0.2	6.4 ± 0.7	5.5 ± 0.1
C22:1 (9)	3.8 ± 0.2	1.3 ± 0.2	2.5 ± 0.5	0.4 ± 0.4	0.4 ± 0.4	0.3 ± 0.3
C22:6(3)	0.7 ± 0.7	nil*	nil*	nil*	5.2 ± 0.6	5.0 ± 0.5
C24:1 (9)	1.7 ± 0.8	0.9 ± 0.1	nil*	5.4 ± 5.4	2.5 ± 0.7	1.2 ± 0.3
Σ SFA	126.4 ± 30.6	85.3 ± 13.2	35.1 ± 8.2	38.8 ± 3.9	153.4 ± 23.6	130.1 ± 46.4
Σ MUFA	49.1 ± 10.1	32.6 ± 5.0	78.0 ± 19.5	72.2 ± 9.7	311.3 ± 52.8	279.6 ± 104.3
Σ PUFA	135.9 ± 40.2	80.3 ± 16.2	181.2 ± 63.5	114.5 ± 26.4	125.4 ± 18.7	131.2 ± 45.0
Σ n-6	132.8 ± 41.6	80.0 ± 16.1	178.2 ± 62.9	113.8 ± 26.1	109.4 ± 17.3	111.7 ± 41.5
Σ n-3	3.1 ± 1.8	0.3 ± 0.3	3.0 ± 0.6	0.6 ± 0.6	16.0 ± 2.0	19.5 ± 4.0
TOTAL	447 ± 121	278 ± 50	475 ± 154	340 ± 59	716 ± 113	672 ± 235

nil* = ≤0.04 μg/ml

TABLE VII.AP1: Fatty acid composition of plasma PL, CE and TG isolated from subjects before (harvest) and after (harvest) HDCT expressed as μg/ml plasma calculated using 17:0 (10 μg; PL and CE) 15:0 (10 μg; TG) as the standards. Data are expressed as means ± SEM (n =3).

Appendix VII.AP2

	PL		CE		TG	
Fatty acid	Harvest	Discharge	Harvest	Discharge	Harvest	Discharge
	(% fatty acids)		*(% fatty acids)*		*(% fatty acids)*	
C14:0	0.1 ± 0.1	nil*	0.4 ± 0.3	0.7 ± 0.5	0.6 ± 0.4	0.7 ± 0.1
C16:0	24.5 ± 1.6	27.0 ± 1.4	10.8 ± 1.0	15.0 ± 2.9	18.8 ± 2.3	20.9 ± 1.2
C16:1	0.5 ± 0.3	0.5 ± 0.3	2.3 ± 0.4	3.0 ± 0.5	2.7 ± 0.4	3.3 ± 0.4
C17:1	0.2 ± 0.1	0.3 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.3 ± 0.03
C18:0	16.2 ± 1.0	16.3 ± 1.0	1.3 ± 0.2	2.8 ± 1.5	4.2 ± 0.5	3.9 ± 0.3
C18:1	13.5 ± 0.7	14.6 ± 0.7	24.5 ± 2.1	26.9 ± 1.9	45.8 ± 2.5	48.5 ± 0.7
C18:2 (6)	22.7 ± 1.5	18.9 ± 1.3	49.6 ± 2.7	38.1 ± 3.8	16.5 ± 1.6	14.7 ± 0.3
C18:3 (6)	nil*	0.1 ± 0.1	0.7 ± 0.2	0.5 ± 0.5	0.1 ± 0.1	0.3 ± 0.1
C18:3 (3)	0.5 ± 0.2	0.1 ± 0.1	0.7 ± 0.01	0.2 ± 0.2	1.2 ± 0.4	0.9 ± 0.2
C20:0	0.1 ± 0.1	nil*	nil*	nil*	0.1 ± 0.1	0.1 ± 0.1
C20:1 (9)	0.3 ± 0.02	0.1 ± 0.1	nil*	nil*	0.4 ± 0.2	0.4 ± 0.1
C20:2 (6)	1.1 ± 0.6	0.4 ± 0.1	0.4 ± 0.2	0.1 ± 0.1	2.5 ± 1.4	1.3 ± 0.2
C20:3 (6)	4.1 ± 0.8	3.3 ± 0.8	0.8 ± 0.1	0.5 ± 0.3	0.2 ± 0.1	0.2 ± 0.08
C20:4 (6)	13.2 ± 1.5	17.2 ± 2.9	6.9 ± 0.7	9.8 ± 2.9	1.6 ± 0.3	2.1 ± 0.6
C20:5 (3)	0.6 ± 0.6	0.1 ± 0.1	0.4 ± 0.2	0.1 ± 0.1	2.1 ± 1.3	1.0 ± 0.2
C22:1 (9)	1.4 ± 0.4	0.7 ± 0.0	1.0 ± 0.3	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
C22:6(3)	0.4 ± 0.4	nil*	nil*	0.1 ± 0.1	1.7 ± 1.1	0.9 ± 0.3
C24:1 (9)	0.5 ± 0.1	0.5 ± 0.1	nil*	2.1± 2.1	1.0 ± 0.7	0.2 ± 0.04
Σ SFA	41.0 ± 0.8	43.4 ± 0.9	12.6 ± 1.1	18.7 ± 5.1	23.9 ± 2.0	26.0 ± 0.9
Σ MUFA	16.4 ± 1.4	16.7 ± 1.2	27.9 ± 2.5	32.1 ± 0.4	50.1 ± 2.2	52.6 ± 1.0
Σ PUFA	42.6 ± 2.0	39.9 ± 1.9	59.5 ± 3.3	49.2 ± 5.4	26.0 ± 3.3	21.3 ± 0.2
Σ n-6	41.2 ± 3.1	39.8 ± 1.9	58.4 ± 3.5	48.9 ± 5.3	21.0 ± 1.6	18.5 ± 0.3
Σ n-3	1.5 ± 1.2	0.1 ± 0.1	1.1 ± 0.2	0.3 ± 0.3	5.0 ± 1.5	2.8 ± 0.3

nil* = ≤0.04 μg/ml

TABLE VII.AP2: Fatty acid composition of plasma PL, CE and TG isolated from subjects before (harvest) and after (harvest) HDCT expressed as μg/ml plasma calculated using 17:0 (10 μg; PL and CE) 15:0 (10 μg; TG) as the standards. Data are expressed as means ± SEM (n =3).

Appendix VIII.AP1

Fatty acid	*Pre FO*	*Post FO*	*Pre Placebo*	*Post Placebo*
		(% fatty acids)		
C 14:0	0.4 ± 0.2	0.5 ± 0.2	0.5 ± 0.0	0.5 ± 0.0
C 15:0	0.3 ± 0.0	0.4 ± 0.1	0.4 ± 0.0	0.3 ± 0.0
C 15:1	0.5 ± 0.0^{ab}	0.5 ± 0.1^{a}	0.3 ± 0.1^{b}	0.3 ± 0.0^{b}
C 16:0	29.4 ± 0.5	30.8 ± 0.9	29.0 ± 1.1	29.8 ± 1.1
C 16:1(5)	0.4 ± 0.1	0.3 ± 0.0	0.4 ± 0.0	0.4 ± 0.1
C 18:0	13.1 ± 0.4	14.3 ± 0.9	13.0 ± 0.6	11.8 ± 0.4
C 18:1(9)	12.0 ± 0.7^{ab}	10.4 ± 0.6^{a}	12.4 ± 0.8^{ab}	13.3 ± 1.2^{b}
C 18:1(7)	1.9 ± 0.1	2.2 ± 0.1	2.3 ± 0.2	2.2 ± 0.2
C18:2 (6)	18.0 ± 0.8^{a}	15.2 ± 0.6^{b}	17.0 ± 0.9^{ab}	17.8 ± 1.1^{a}
C 19:0	1.1 ± 0.1	1.3 ± 0.2	1.4 ± 0.1	1.3 ± 0.3
C 18:3(3)	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.1
C 20:0	0.8 ± 0.1^{a}	0.9 ± 0.2^{ab}	1.5 ± 0.1^{b}	1.1 ± 0.3^{ab}
C 20:1(9)	nil*	0.3 ± 0.2	nil*	0.1 ± 0.1
C 20:1(7)	0.2 ± 0.1	0.3 ± 0.2	0.1 ± 0.0	0.3 ± 0.1
C 20:2(6)	0.6 ± 0.1^{a}	0.3 ± 0.0^{b}	0.5 ± 0.1^{ab}	0.4 ± 0.1^{ab}
C 20:3(9)	2.5 ± 0.3a	1.3 ± 0.2b	2.1 ± 0.2a	2.1 ± 0.2a
C 20:3(6)	1.2 ± 0.1	1.4 ± 0.2	1.6 ± 0.1	1.6 ± 0.3
C 20:4(6)	8.7 ± 0.4	7.9 ± 0.4	8.6 ± 0.4	7.6 ± 0.4
C 20:5(3)	0.7 ± 0.2^{a}	3.8 ± 0.6^{b}	0.5 ± 0.1^{a}	0.5 ± 0.1^{a}
C 22:0	1.1 ± 0.1	1.4 ± 0.2	1.6 ± 0.1	1.5 ± 0.3
C 22:1(9)	0.5 ± 0.1	0.6 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
C 23:0	1.1 ± 0.1	1.2 ± 0.2	1.4 ± 0.1	1.4 ± 0.3
C 22:4(6)	0.2 ± 0.0^{a}	0.1 ± 0.1^{b}	0.3 ± 0.0^{a}	0.2 ± 0.1^{a}
C 22:5(6)	0.3 ± 0.1	nil*	0.3 ± 0.1	0.3 ± 0.1
C 22:5(3)	0.6 ± 0.1^{a}	1.0 ± 0.1^{b}	0.7 ± 0.0^{c}	0.6 ± 0.0^{a}
C 24:0	0.9 ± 0.1	1.0 ± 0.2	1.0 ± 0.1	1.0 ± 0.2
C 22:6(3)	2.9 ± 0.3	4.1 ± 0.5	3.2 ± 0.5	2.8 ± 0.3

nil* = ≤0.04%

TABLE VIII.AP1: Fatty Acid content of plasma PL expressed as percent of total fatty acids before and after 14 days of supplementation with fish oil (FO, n=13) or Placebo oil (n=10). Data are expressed as means ± SEM. Significant differences were determined using a repeated measures ANOVA and values within a row not sharing common superscripts are significantly different ($p<0.05$).

Appendix IX.1

Method Development for Fatty Acid Incorporation into Neutrophil Phospholipids

A. ***Using a purified neutrophil suspension after fatty acid incorporation into whole blood***: Fatty acids were used at a concentration of 1 mg/ml as this has shown to result in fatty acid incorporation into whole blood cultures (Bellinati-Pires et al., 1993)and is similar to the total fatty acid concentrations found in plasma of the burn subjects. Fatty acids were prepared using the stock solutions described in Section IX.B and incubated with HBSS + 0.1% fatty acid free BSA for 1 hour as described in the methods. Whole blood (8 ml) was incubated at a 4:1 ratio (Bellinati-Pires et al., 1993) with the fatty acid suspensions. [^{3}H]-arachidonic acid (100 µl/ml; 20µl) was added to each tube. Samples were rotated on a rotor for 1, 2, 4, 8 and 24 hours in humidified atmosphere at 37°C. After each incubation, neutrophils were isolated using Ficoll-Hypaque (1119/1077 described in Chapter III) from 2 ml of each fatty acid/whole blood suspension. The neutrophil band was removed with a plastic transfer pipette and washed twice in HBSS. Cells were counted and adjusted to 1 x 10^6 cells/ ml. One ml was added to scintillation vials containing Ecolite® (2 ml) and counted in a Beckman counter to measure incorporation of [^{3}H]-arachidonic acid label into cells.

** this method resulted in low neutrophil yield (Table IX.AP1).

B. ***Using whole blood deplete of erythrocytes***: Red blood cells were lysed from whole blood (5 ml) using warmed lysis buffer and inverting several times. Cells were spun at 1250 rpm for 10 minutes. Supernatant was removed and cells were gently washed twice with HBSS. After the last wash, cells were suspended in 1 ml HBSS and counted using trypan blue and crystal violet staining to assess both viability and percent neutrophils in the suspension.

Neutrophils (1 x 10^6) were added to each tube of fatty acids prepared in 1 ml BSA as described in Section IX.B. [^{3}H]-arachidonic acid (20 µl of a 10 µl/ml stock, 40000 cpm) was added to each tube. Tubes were incubated for 1, 2, 4, 8 and 24 hours. At each timepoint, cells were pelleted by centrifuging at 1500 for 2 minutes. Supernatants were collected and added to scintillation vials containing Ecolite® (2 ml) and samples were counted in a Beckman counter to measure disappearance of radiaoactive label from the supernatant.

**This procedure resulted in disappearance of the label, however, the cell culture contained both lymphocytes and neutrophils and these two cell types may vary in their ability to take up fatty acids (Figure IX.AP1). The inconsistencies in fatty acid incorporation observed at the different timepoints (non-linear pattern) were thought to be due to higher numbers of lymphocytes in those suspensions.

C. ***Purifying Neutrophils prior to fatty acid incorporation:*** Neutrophils were isolated from whole blood using Ficoll Hypaque gradient centrifugation as described in Section III.B. The neutrophil band was removed and washed twice with HBSS. Cells were counted and adjusted to 1 x 10^6 cells/ ml. Cells (1 x 10^6) were incubated with the fatty acid suspension (10^{-7} M) with or without [^{3}H]-arachidonic acid for 2, 4 and 6 hours in a shaking water bath set at 37°C. Following incubation, tubes were spun for 5 minutes at 1250 rpm and supernatants removed. Cells were washed once with HBSS and counted using trypan blue exclusion. Cells (1 x 10^6) were added to scintiallation vials containing Ecolite® and counted using a Beckman counter to measure incorporation of label into neutrophils. Cells incubated without ^{3}[H]-AA were used to measure functional parameters of neutrophils using flow cytometry.

**Gradient isolated neutrophils provided a more accurate cell count and more consistent label incorporation. Oxidative burst was retained up to 8 hours when gradient isolated neutrophils were used (Figure IX.AP2).

Table IX.AP1

Time of incubation	AA	EPA	LA	Buffer
	(Cells/ml)			
1 hour	3.4 ± 3.3	1.4 ± 0.4	2.1 ± 1.0	2.4 ± 0.71
2 hour	0.8 ± 1	0.7 ± 0.9	0.5 ± 0.6	2.0 ± 2.3
4 hour	0.8 ± 0.4	0.8 ± 0.1	0.2 ± 0.0	2.2 ± 0.6
8 hour	0.4 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	1.1 ± 0.2

TABLE IX.AP1: Data is shown as mean ± SD (n=2). Number of neutrophils obtained from ficoll-hypaque gradient centrifugation after fatty acid incubation with whole blood. Minimum number of neutrophils required to perform the planned assays was 2 x 10^6 cells.

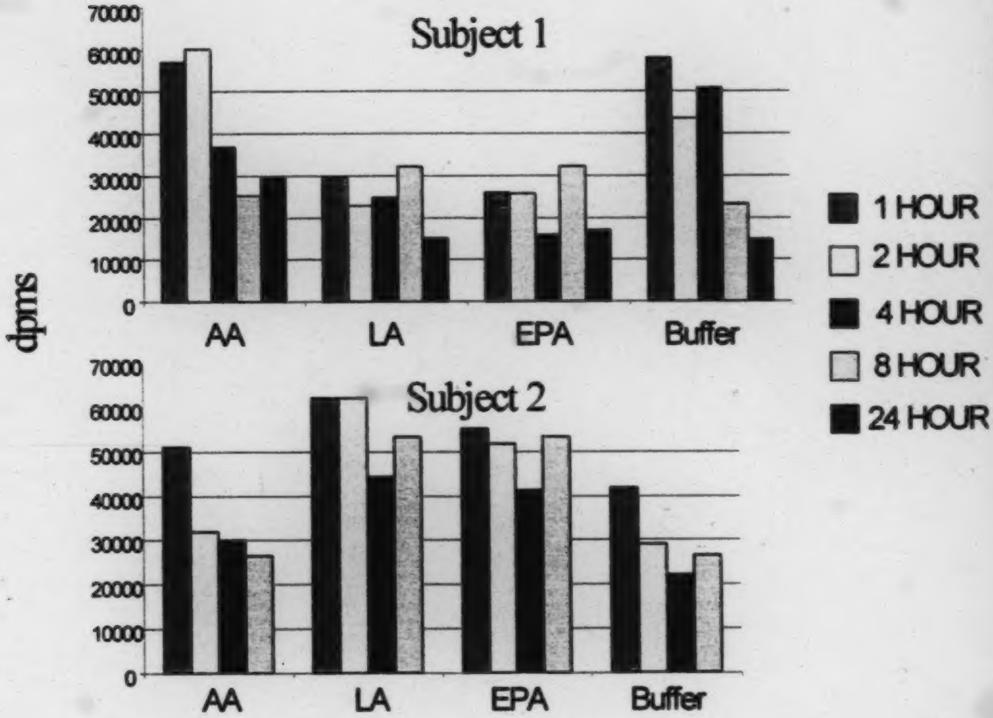


FIGURE IX.AP1: Columns represent means and data is shown as 2 different subjects. Measurement of the disappearance of $[^3H]$-arachidonic acid in supernatants of cultures of whole blood deplete of red blood cells after incubation with fatty acids.

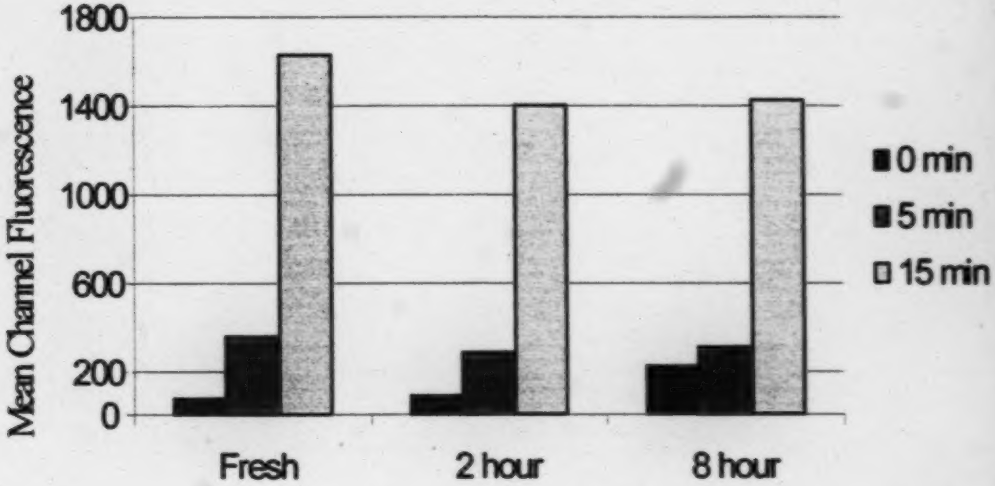


FIGURE IX.AP2: Gradient isolated neutrophils were cultured with fatty acids and oxidative burst was measured after 5 and 15 minutes stimulation with PMA *in vitro*. Fresh refers to whole blood deplete of red blood cells that were stimulated in the same way. Columns represent the data from one subject.

D. References

Bellinati-Pires R, Waitzberg DL, Salgado, MM, Carneiro-Sampaio MM. Functional alterations of human neutrophils by medium-chain triglyceride emulsions: evaluation of phagocytosis, bacterial killing, and oxidative activity. J Leukoc Biol 53: 404-410, 1993.